(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 March 2003 (27.03.2003)

PCT

(10) International Publication Number
WO 03/025137 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/29449

(22) International Filing Date:
17 September 2002 (17.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/323,450 18 September 2001 (18.09.2001) US

(71) Applicants *(for all designated States except US)*: **IRM, LLC** [US/—]; P.O. Box HM 2899, Hamilton HM LX (BM). **THE SCRIPPS RESEARCH INSTITUTE** [US/US]; 10550 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LIAO, Jiayu** [CN/US]; 5032 Ashberry Road, Carlsbad, CA 92008 (US). **DING, Sheng** [CN/US]; 7867 Avenida Navidad, #242, San Diego, CA 92122 (US). **SCHULTZ, Peter, G.** [US/US]; 1650 La Jolla Ranch Road, La Jolla, CA 92037 (US).

(74) Agents: **QUINE, Jonathan, Alan** et al.; Quine Intellectual Property Law Group, P.C., P.O. Box 458, Alameda, CA 94501 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/025137 A2

(54) Title: SWEET TASTE RECEPTORS

(57) Abstract: This invention provides novel genes and polypeptides of the sweet receptor family, methods for production of the polypeptides, methods for screening compounds that specifically bind to and/or modulate the activity of these polypeptides; and antibodies specific for the polypeptides.

SWEET TASTE RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Non-Provisional of USSN 60/323,450, "Sweet Taste Receptors" by Liao and Schultz, filed 09/18/2001, which is incorporated herein by reference in its entirety. The subject application claims priority to and benefit of USSN 60/323,450.

FIELD OF THE INVENTION

[0002] This invention relates to novel sweet receptor nucleic acids and polypeptides. In particular, the invention relates to polypeptides that are homologous to other sweet receptors, nucleic acids encoding the polypeptides, vectors and host cells comprising the nucleic acids and antibodies that specifically bind to the polypeptides. The invention also relates to recombinant methods for producing the polypeptides and methods for identifying compounds that bind to and/or modulate the activity of the polypeptides.

BACKGROUND OF THE INVENTION

[0003] In mammals, there are three chemosensory systems (taste, olfactory and vomeronasal perceptions) that function to convert external chemical signals to specific neuronal activities. These neuronal signals are then integrated in different regions of brain and the output of these signals affect the organism's various innate behaviors, ranging from aversion and attraction to food or small volatile chemicals to reproductive actions. Among these chemosensory systems, taste perception provides immediate valuation of nutrients. Although the molecular universe of tastants consists of diverse chemical structures such as ions, small organic molecules, proteins, carbohydrates, amino acids, and lipids, it is generally believed that mammals have five basic taste modalities: sour, salty, bitter, sweet, and umami (glutamate) as described, e.g., in Lindemann, *Physiol. Rev.* 76:718-766, 1996;

Kinnamon et al., *Annu. Rev. Physiol.* 54:715-731, 1992; and Gilbertson et al., *Curr. Opin. Neurobiol.* 10: 519-527, 2000.

[0004] The sensation of taste is initiated by the interaction of tastants with their receptors in the taste cells, which are clustered in onion-shape taste buds embedded within the lingual epithelium in tongue and palate as described, e.g., in Lindemann, *supra.* On the tongue, taste buds are topographically distributed into papillae in different locations of tongue. Fungiform papillae are located at the front of the tongue and contain a small number of taste buds; foliate papillae, containing dozens of taste buds, are localized along the posterior lateral edge of the tongue; and at the back of the tongue, circumvallate papillae contain thousands of taste buds. Classical physiological studies have found that fungiform papillae are sensitive to sweet, foliate papillae are sensitive to sour and bitter, and circumvallate papillae are particularly sensitive to bitter.

[0005] Each taste modality is thought to be mediated by distinct cell surface receptors that are expressed in a subset of taste cells. Electrophysiological and biochemical studies suggest that salty and sour tastants signal through Na^+ and H^+ membrane channels as described, e.g., in Heck et al. *Science* 223: 403-405, 1984; Avenet et al., *J. Memb. Biol.* 105:245-255, 1988, Doolin et al., *J. Gen. Physiol.* 107:545-554, 1996; Formaker et al., *Am. J. Physiol* 255:1002-1007, 1988; Kinnamon et al. *Proc. Natl. Acad. Sci. USA* 85:7023-7027, 1988; and Gilbertson et al., *J. Gen. Physiol.* 100:803-824, 1992. In contrast, bitter, sweet, and umami taste transduction are believed to involve G protein-coupled receptors (GPCR).

[0006] GPCRs are a class of seven-transmembrane proteins which transduce an extracellular signal, i.e., ligand binding to receptor, into a cellular response. Upon ligand binding to a GPCR, the GPCR activates an intracellular guanine nucleotide protein known as G-protein (guanine nucleotide binding protein), which mediates a response to the extracellular signal. G-proteins are heterotrimeric proteins composed of an alpha, beta and gamma subunit. The activated G protein alters the activity of various cellular effector enzymes (e.g., adenylate cyclase and phosphodiesterase), which in turn alters the levels of various second messengers (e.g., cAMP, cGMP, and inositol triphosphate (IP_3)).

[0007] Experiments with the bitter substance, denatonium, have shown that the secondary messages, cAMP and IP_3, are induced in response to bitter stimuli as described, e.g., in Spielamn et al., *Am. J. Physiol.* 270:C926-C931, 1996; and Ruiz-Avila et

al., *Nature* 376:80-85, 1995. Other studies have revealed that gustducin, a G protein expressed in subpopulation of taste buds, can activate phosphodiesterase (PDE) and thereby decrease cNMP levels in response to bitter stimuli as described, e.g., in Ruiz-Avila et al, *supra*; and Hoon et al., *Biochem. J.* 309:629-636, 1995. These secondary messages, which are generally involved in G protein signaling, are consistent with the involvement of GPCRs in taste transduction. Sweet substances have also been shown to cause the elevation of the secondary messages, cAMP and IP_3, presumably in response to activation of G protein-coupled receptor cascades by Gs protein as described, e.g., in Striem et al., *Biochem. J.* 260:121-126, 1989; and Bernhardt et al., *J. Physiol.* 490:325-336, 1996. The involvement of G proteins in bitter and sweet transduction is also supported by the discovery that mice with a null allele of gustducin have an impaired ability to detect bitter and sweet substances as described, e.g., in Wong et al., *Nature* 381:796-800, 1996.

[0008] The involvement of G-protein coupled receptors in taste transduction has recently been confirmed by the discovery of three families of GPCRs expressed in mammalian taste bud cells, a number of which have been shown to be activated by bitter and glutamate tastants as described, e.g., in Firestein, *Nature* 404:552-553, 2000. A splice variant of a metabotropic glutamate receptor was cloned from rat taste bud and was shown to respond to monosodium L-glutamate when expressed in heterogonous cells as described, e.g., in Chaudharri et al., *Nature Neurosci.* 3:113-119, 2000. Two additional candidate taste receptors, T1R1 and T2R2, have been isolated from rat taste bud, and show distant homology with putative pheromone receptor V2Rs and metabotropic glutamate receptors, as described in Hoon et al., *Cell* 96:541-552, 1999. T1R1 and T2R2 were postulated to function as sweet and bitter receptors, respectively, based on their topographic distribution in the tongue as described, e.g., in Hoon et al., *supra*, 1999. Searches of the human and mouse genomes have identified another family of taste receptors (T2Rs) containing approximately 25 members as described, e.g., in Adler et al., Cell 100:693-702, 2000; and Matsunami et al., *Nature* 404:601-603, 2000. One receptor in this family, mT2R5, is specifically activated by the bitter substance cycloheximide, while the human hT2R4 and mouse mT2R8 respond to denatonium as described, e.g., in Chandrashekar et al., *Cell* 100:703-711, 2000.

[0009] Over the past few years, much effort has been directed toward the development of various sweeteners that interact with taste receptors to mimic natural sweet

taste stimulants. See, Robert H. Cagan, Ed., *Neural Mechanisms in Taste*, Chapter 4, CRC Press, Inc., Boca Raton, FL,1989. Examples of sweeteners that have been developed to mimic sweet tastes are saccharin (an anhydride of o-sulfimide benzoic acid), monellin (a protein), aspartame (a peptide composed of aspartic acid and methyl ester of phenylalanine) and the thaumatins (also proteins). Many sweeteners developed to date are not suitable as food additives, however, because they are uneconomical, high in calories, carcinogenic or lose their sweetness when exposed to elevated temperatures for long periods, rendering them unsuitable for use in most baking applications.

[0010] Development of new sweeteners that mimic sweet (and other) tastes has been limited by a lack of knowledge of the taste cell proteins responsible for transducing the sweet taste modalities. Accordingly, the identification of new sweet taste receptors would enable the identification of the natural ligands, i.e., natural sweet tastants, of these proteins and the design of novel sweeteners that mimic sweet taste perception. The present invention fulfills these and other needs.

SUMMARY OF THE INVENTION

[0011] The present invention relates members of the sweet receptor family, in particular human sweet receptor 1 (hT1R1), human sweet receptor 2 (hT1R2) and human sweet receptor 3 (hT1R3) nucleic acids and polypeptides, vectors and host cells comprising the nucleic acids, antibodies to the polypeptides, and methods for producing the polypeptides. In another aspect, the present invention relates to methods for identifying agents that bind to and/or modulate the activity of these polypeptides, e.g., use of the polypeptides (e.g., when present in biological materials) as sensor or assay components to detect molecules that are perceived as sweet and/or that provide for glutamate (umami) detection. The invention also provides for rescue of sweet and/or umami taste function in cells that are defective in expression of hT1R1, hT1R2 or hT1R3, e.g., by expressing the polypeptides of the invention in the cells, e.g., from a recombinant construct. This cell rescue can be performed in vitro (e.g., in cell culture) or in vivo (e.g., in mammalian taste buds).

[0012] Accordingly, in a first aspect, the invention provides isolated or recombinant polypeptides (e.g., that comprises hT1R1, hT1R2 or hT1R3 function). These polypeptides can be characterized in any of a variety of related ways. For example, the

polypeptides of the invention can include an amino acid sequence or subsequence that is at least 75% identical to an hT1R1 polypeptide (e.g., SEQ ID NO. 1), an hT1R2 polypeptide (e.g., SEQ ID NO.4), and/or an hT1R3 polypeptide (e.g., SEQ ID NO.7), e.g., as determined by BLASTP using default parameters (or another comparison algorithm or via manual alignment). Similarly, the polypeptides of the invention can include an amino acid sequence or subsequence that comprises one or more domains of an hT1R1 polypeptide, an hT1R2 polypeptide, or an hT1R3 polypeptide, e.g., where the hT1R1 polypeptide, the hT1R2 polypeptide, or the hT1R3 polypeptide comprises an amino acid sequence such as those of hT1R1, hT1R2 and/or hT1R3 (e.g., SEQ ID NO. 1, SEQ ID NO.4 and SEQ ID NO.7, respectively). In a related aspect, the polypeptides of the invention can include an amino acid sequence or subsequence that is at least 75% identical to a domain encoded by hT1R1, hT1R2 and/or hT1R3, (e.g., SEQ ID NO. 1, SEQ ID NO.4 or SEQ ID NO.7, respectively), e.g., as determined by BLASTP using default parameters, where the domain includes: an amino-terminal extracellular domain; an extracellular domain located between TM2 and TM3, between TM4 and TM5, or between TM6 and TM7; a transmembrane (TM) domain; an intracellular domain located between TM1 and TM2, between TM3 and TM4, or between TM5 and TM6; and/or a carboxyl-terminal intracellular domain. Any polypeptide of the invention optionally includes one or more of these domains. The polypeptides of the invention can also be defined by immunoreactivity, e.g., the polypeptides of the invention can include an amino acid sequence or subsequence that is specifically bound by an antibody that specifically binds to an amino acid such as hT1R1, hT1R2 and/or hT1R3 (e.g., SEQ ID NO. 1, SEQ ID NO.4, and/or SEQ ID NO.7, respectively) where the antibody is not specifically bound by an amino acid from the corresponding mouse or rat homologues (e.g., as represented at SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 8, and/or SEQ ID NO. 9). Examples of polypeptides of the invention include the polypeptides encoded by amino acid sequences or subsequences that are encoded by SEQ ID NO. 10, SEQ ID NO. 11 and/or SEQ ID NO 12, and/or complementary sequences thereof. In general, the polypeptides of the invention can also be defined with respect to the nucleic acids that encode them, e.g., polypeptides of the invention can include an amino acid sequence or subsequence that is encoded by a first nucleic acid that specifically hybridizes to a second nucleic acid, wherein the second nucleic acid is a nucleic acid that encodes hT1R1,

hT1R2 and/or hT1R3 (e.g., SEQ ID NO. 10, SEQ ID NO. 11 and/or SEQ ID NO 12), or a complement thereof, under stringent conditions, where the first nucleic acid hybridizes to the second nucleic acid under the stringent conditions with at least 5x an affinity that the first nucleic acid hybridizes to a third nucleic acid that encodes a mouse or rat homologue, e.g., an mT1R1 nucleic acid, an rT1R1 nucleic acid, an mT1R2 nucleic acid, an rT1R2 nucleic acid, an mT1R3 nucleic acid and/or a rT1R3 nucleic acid. Also encompassed within the polypeptides of the invention are any and all amino acid sequences or subsequences corresponding to a conservative variation of any of the amino acid sequences or subsequences noted above, e.g., an amino acid sequence such as SEQ ID NO. 1, SEQ ID NO.4, SEQ ID NO.7, or a conservative variation thereof.

[0013] In one aspect the polypeptide of the invention is a mature polypeptide, e.g., a mature hT1R1 protein, a mature hT1R2 protein, or a mature hT1R3 protein, e.g., a protein having an activity of the hT1R1, hT1R2 or hT1R3 protein. The polypeptide can be, e.g., a monomer, a homomultimer or a heteromer. For example, the polypeptide can be a homomultimer or a heteromer that includes more than one polypeptide, e.g., as shown by SEQ ID NO. 1, SEQ ID NO.4, and/or SEQ ID NO.7 (hT1R1, hT1R2 and hT1R3, respectively), or a conservative variation thereof. Also provided by the invention are isolated polypeptides that include one or more domains of an hT1R1, hT1R2, or hT1R3 polypeptide.

[0014] In addition to the polypeptides noted above, methods for producing a recombinant or isolated polypeptide are also provided. For example, the methods can include growing a cell in culture comprising an expression vector encoding a recombinant or isolated polypeptide as described above, under conditions suitable for expression of the isolated or recombinant polypeptide. The polypeptide is then purified, e.g., such that the polypeptide is enriched at least 5X (and typically 50X, 100X, 1000X or more) as compared to the polypeptide present in the culture. The resulting isolated or recombinant polypeptide made by this method is also a feature of the invention.

[0015] Nucleic acids, e.g., isolated or recombinant nucleic acids, are also a feature of the invention. For example, a nucleic acid that encodes any of the preceding polypeptides (e.g., SEQ ID NO. 1, SEQ ID NO. 4, and SEQ ID NO. 7, or a conservative variation thereof) is optionally a feature of the invention. In one class of embodiments, the

nucleic acid encodes a substantially full-length a polypeptide, and/or is capable of rescuing a function of a mutant or recombinant cell that is defective with respect to hT1R1, hT1R2 or hT1R3 (e.g., where the cell is a deletion mutant with respect to hT1R1, hT1R2 and/or hT1R3). Exemplar nucleic acids of the invention include those represented at SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, (hT1R1, hT1R2 and hT1R3 nucleic acids, respectively) and/or complementary sequences thereof. The nucleic acid optionally includes a DNA (e.g., a gDNA, a cDNA or a DNA cloning or expression vector), or an RNA (e.g., an mRNA or RNA cloning or expression vector).

[0016] In one related aspect, the invention includes an hT1R2 nucleic acid that hybridizes under stringent conditions to a first nucleic acid, e.g., that includes the first two exons (e.g., nucleotides 1-483) from nucleotide sequence of SEQ ID NO. 11, or to a complement thereof, wherein the stringent conditions are selected such that the hT1R2 nucleic acid preferentially hybridizes to the first nucleic acid as compared to a mT1R2 nucleic acid or complement thereof, or to an rT1R2 nucleic acid or complement thereof. For example, the hT1R2 nucleic acid can encode a hT1R2 polypeptide comprising the sequence set forth at SEQ ID NO: 4. Here again, the nucleic acid optionally encodes a substantially full length hT1R2 polypeptide and can be a DNA or RNA (e.g., a gDNA, a cDNA or a DNA cloning or expression vector), or an RNA (e.g., an mRNA or RNA cloning or expression vector). For example, the hT1R2 nucleic acid can include or be coded within an expression vector.

[0017] The present invention also provides antibodies, e.g., antibodies or fragments thereof which specifically bind the isolated or recombinant polypeptides described above. For example, the antibody fragment can be an Fab or F(ab')2 fragment, the antibody can be a monoclonal or polyclonal antibody, or the like. Optionally, the antibody can be a discriminatory antibody that specifically hybridizes to a polypeptide as noted above, but which does not specifically bind to a rat or mouse homologue protein, e.g., mT1R1, mT1R2, mT1R3, rT1R1, rT1R2 or rT1R3.

[0018] As noted above, in one aspect, expression vectors that encode the polypeptides noted above are provided. Similarly, cells that include the expression vectors are a feature of the invention. In addition, biosensors comprising the polypeptides are also a feature of the invention.

[0019] In one aspect, the invention includes a database and/or a computer-readable medium comprising a character string that represents any polypeptide, nucleic acid, cell, vector, antibody or other material of the invention that is noted herein. Optionally, the database or computer readable medium is coupled to one or more instruction set, software package, network, internet, intranet, user input, user-viewable output, computer, or other feature or component that transmits, manipulates, reads or otherwise acts upon the database or computer-readable medium.

[0020] The invention also provides methods of identifying compounds which bind to and/or modulate an activity of the isolated or recombinant polypeptides noted above. In the methods, a biological sample comprising the isolated or recombinant polypeptide is contacted with a test compound. binding and/or modulation of the activity of the polypeptide by the compound is then detected, thereby identifying a compound which binds to and/or modulates the activity of the polypeptide. The detection of binding or activity can take any of a wide variety of forms, e.g., detecting binding of an antibody to the isolated or recombinant polypeptide, or detecting a signal produced by the isolated or recombinant polypeptide. In addition to detection of activity of the polypeptides noted above, cells or other biological materials that include endogenous hT1R1, hT1R2 or hT1R3 can be used in the methods (e.g., cultures of cells derived from taste buds, or the like). Optionally, such materials and methods do not include testing cells in a mammal, e.g., in a human.

[0021] Examples of signals that can be detected include conformation-dependent signals, e.g., where a conformation of the isolated or recombinant polypeptide is modified by binding of the test compound to the isolated or recombinant polypeptide. Detecting binding can include, e.g., one or more of: a Ca^{2+} flux assay, a cAMP assay, a GTPgammaS binding assay, a melanophore assay, a phospholipase C assay, a beta-arrestin FRET assay, and a transcriptional reporter assay. Where detection includes measuring a signal from a transcriptional reporter assay (e.g., detection of a reporter gene (e.g., CAT activity) coupled to a response element that is controlled by a second messanger activated by hT1R1, hT1R2 and/or hT1R3, or a multimer thereof), common response elements that can be detected include: a CRE, a SRE, an MRE, a TRE, an NFAT, and/or an NFkB-response element.

[0022] The biological sample can be in any of a variety of configurations, e.g., cells which express the recombinant polypeptide, biosensors (liquid or solid phase), a Chem-FET, a cell extract; a membrane preparation comprising the protein of interest or another material comprising the proteins noted herein, or the like.

[0023] The invention also provides methods of rescuing cells that have altered or missing T1R1, T1R2, or T1R3 function (e.g., due to deletion or other mutation of genes relevant to such function). In the methods, a nucleic acid that encodes the recombinant polypeptide noted above is introduced into a cell and expressed, thereby providing hT1R1, hT1R2, or hT1R3 function to the cell. The cell can, e.g., be in cell culture, in a tissue, in a taste bud, in a mammal (e.g., a human), or the like.

[0024] The invention also includes kits, e.g., comprising a polypeptide, nucleic acid, vector, cell or antibody as noted above and further including, e.g., instructional materials in the use of the polypeptides or nucleic acids, e.g., in the methods herein, packaging materials, containers for holding other kit elements, and the like.

BRIEF DESCRIPTION OF THE FIGURES

[0025] **Figure 1** is a sequence alignment between human, mouse and rat sweet receptor sequences. Three putative human sweet receptor proteins are aligned with three mouse T1Rs (mT1R1, mT1R2, mT1R3), and two rat T1Rs (rT1R1, rT1R2) using ClustalW. Horizontal bars indicate seven-transmembrane domains for GPCRs as predicted using hT1R1 protein; potential signal peptides for hT1R1-3 are boxed. Identical amino acids are boxed in black, while conserved amino acids are boxed in gray. As shown, the three human sweet receptors are related to mouse and rat T1Rs.

[0026] **Figures 2A-2C** provide nucleotide sequences of hT1R1, hT1R2 and hT1R3 cDNAs. Figure 2A shows the nucleotide sequence of the hT1R1 cDNA. Figure 2B shows the nucleotide sequence of the hT1R2 cDNA. Figure 2C shows the nucleotide sequence of the hT1R3 cDNA.

[0027] **Figure 3** is a schematic showing the structure/ location of the three sweet receptor genes (clustered in human chromosome 1). (Top) Chromosome mapping studies were carried out using the NCBI human genome search interface with the distance to the end of the chromosome shown in kilobases (K) (not to scale). The chromosome locations of two genes (T1R2 and T1R3) were determined using two BAC clones

(AL080251 and AL391244, respectively) that are located very close to the two genes. The arrow indicates the span and orientation of the gene. The numbers under the arrows indicate the size of the gene, including introns and exons. The number for T1R2 is approximate because the sequence for the transcriptional start region and first two exons is not available. (Bottom) The distal region of mouse chromosome 4 corresponds to the syntenic region of human 1p36.33. The locations of three mouse T1Rs were obtained from The Jackson Laboratory Mouse Informatics Database.

DETAILED DESCRIPTION

DEFINITIONS

[0028] A "host cell," as used herein, refers to a prokaryotic or eukaryotic cell that contains heterologous DNA that has been introduced into the cell by any means, e.g., electroporation, calcium phosphate precipitation, microinjection, transformation, viral infection, and/or the like.

[0029] A "vector" is a composition for facilitating introduction, replication and/ or expression of a selected nucleic acid in a cell. Vectors include, e.g., plasmids, cosmids, viruses, YACs, bacteria, poly-lysine, etc. A "vector nucleic acid" is a nucleic acid molecule into which heterologous nucleic acid is optionally inserted which can then be introduced into an appropriate host cell. Vectors preferably have one or more origins of replication, and one or more sites into which the recombinant DNA can be inserted. Vectors often have convenient means by which cells with vectors can be selected from those without, e.g., they encode drug resistance genes. Common vectors include plasmids, viral genomes, and (primarily in yeast and bacteria) "artificial chromosomes." "Expression vectors" are vectors that comprise elements that provide for or facilitate transcription of nucleic acids which are cloned into the vectors. Such elements can include, e.g., promoters and/or enhancers operably coupled to a nucleic acid of interest.

[0030] "Plasmids" generally are designated herein by a lower case p preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art. Starting plasmids disclosed herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids by routine application of well known, published

procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art. Moreover, those of skill readily may construct any number of other plasmids suitable for use in the invention. The properties, construction and use of such plasmids, as well as other vectors, in the present invention will be readily apparent to those of skill from the present disclosure.

[0031] The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring nucleic acid, polypeptide, or cell present in a living animal is not isolated, but the same polynucleotide, polypeptide, or cell separated from some or all of the coexisting materials in the natural system, is isolated, even if subsequently reintroduced into the natural system. Such nucleic acids can be part of a vector and/or such nucleic acids or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. A "recombinant nucleic acid" is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling or other procedures. A "recombinant polypeptide" is a polypeptide which is produced by expression of a recombinant nucleic acid. An "amino acid sequence" is a polymer of amino acid residues (a protein, polypeptide, etc.) or a character string representing an amino acid polymer, depending on context. Either the given nucleic acid or the complementary nucleic acid can be determined from any specified polynucleotide sequence.

[0032] The terms "nucleic acid," "DNA sequence" or "polynucleotide" refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in manner similar to naturally occurring nucleotides. A "polynucleotide sequence" is a nucleic acid (which is a polymer of nucleotides (A,C,T,U,G, etc. or naturally occurring or artificial nucleotide analogues) or a character string representing a nucleic acid, depending on context. Either the given nucleic acid or the complementary nucleic acid can be determined from any specified polynucleotide sequence.

[0033] A "subsequence" or "fragment" is any portion of an entire sequence, up to and including the complete sequence. Typically a subsequence or fragment comprises less than the full-length sequence.

[0034] Numbering of a given amino acid or nucleotide polymer "corresponds to numbering" of a selected amino acid polymer or nucleic acid when the position of any given polymer component (amino acid residue, incorporated nucleotide, etc.) is designated by reference to the same residue position in the selected amino acid or nucleotide, rather than by the actual position of the component in the given polymer.

[0035] Proteins and/or protein sequences are "homologous" when they are derived, naturally or artificially, from a common ancestral protein or protein sequence. Similarly, nucleic acids and/or nucleic acid sequences are homologous when they are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. For example, any naturally occurring hT1Rx nucleic acid can be modified by any available mutagenesis method. When expressed, this mutagenized nucleic acid encodes a polypeptide that is homologous to the protein encoded by the original hT1Rx nucleic acid. Homology is generally inferred from sequence similarity between two or more nucleic acids or proteins (or sequences thereof). The precise percentage of similarity between sequences that is useful in establishing homology varies with the nucleic acid and protein at issue, but as little as 25% sequence similarity is routinely used to establish homology. Higher levels of sequence similarity, e.g., 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% or more can also be used to establish homology. Methods for determining sequence similarity percentages (e.g., BLASTP and BLASTN using default parameters) are described herein and are generally available.

[0036] The terms "identical", "sequence identical" or "sequence identity" in the context of two nucleic acid sequences or amino acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. A "comparison window", as used herein, refers to a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are aligned optimally. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2:482; by the alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443; by the search for similarity method of Pearson and

Lipman (1988) *Proc. Nat. Acad. Sci U.S.A.* 85:2444; by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligentics, Mountain View Calif., GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis., U.S.A.); the CLUSTAL program is well described by Higgins and Sharp (1988) *Gene* 73:237-244 and Higgins and Sharp (1989) *CABIOS* 5:151-153; Corpet et al. (1988) *Nucleic Acids Res.* 16:10881-10890; Huang et al (1992) *Computer Applications in the Biosciences* 8:155-165; and Pearson et al. (1994) *Methods in Molecular Biology* 24:307-331. Alignment is also often performed by inspection and manual alignment. In one class of embodiments, the polypeptides herein are at least 70%, generally at least 75%, optionally at least 80%, 85%, 90%, 95% or 99% or more identical to a reference polypeptide, e.g., hT1R1, hT1R2 and/or hT1R3, e.g., as set forth at SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 7 respectively, e.g., as measured by BLASTP (or CLUSTAL, or any other available alignment software) using default parameters. Similarly, nucleic acids can also be described with reference to a starting nucleic acid, e.g., they can be 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more identical to a reference nucleic acid, e.g., hT1R1, hT1R2 and/or hT1R3, e.g., as set forth at SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, respectively, e.g., as measured by BLASTN (or CLUSTAL, or any other available alignment software) using default parameters.

[0037] The terms "substantially identical" nucleic acid or amino acid sequences means that a nucleic acid or amino acid sequence comprises a sequence that has at least 90% sequence identity or more, preferably at least 95%, more preferably at least 98% and most preferably at least 99%, compared to a reference sequence using the programs described above (preferably BLAST) using standard parameters. For example, the BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)). Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the

reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the sequences are substantially identical over at least about 150 residues. In a most preferred embodiment, the sequences are substantially identical over the entire length of the coding regions.

[0038] "Selectively hybridizing" or "selective hybridization" includes hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree that its hybridization to non-target nucleic acid sequences. Selectively hybridizing sequences have at least 50%, or 60% or 70% or 80% or 90% sequence identity or more, e.g., preferably 95% sequence identity, and most preferably 98-100% sequence identity (i.e., complementarity) with each other.

[0039] "Stringent hybridization" conditions or "stringent conditions" in the context of nucleic acid hybridization assay formats are sequence dependent, and are different under different environmental parameters. An extensive guide to hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* Part 1, Chapter 2 "Overview of Principles of Hybridization and the Strategy of Nucleic Acid Probe Assays" Elsevier, New York. Generally, highly stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m)for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m point for a particular nucleic acid of the present invention, this occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. Stringent hybridization conditions are sequence-dependent and will be

different in different circumstances. Longer sequences hybridize specifically at higher temperatures.

[0040] An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (see, Sambrook, supra for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. In general, a signal to noise ratio of 2x (or higher, e.g., 5X, 10X, 20X, 50X, 100X or more) than that observed for control probe in the particular hybridization assay indicates detection of a specific hybridization. For example, the control probe can be a mouse or rate homologue to the relevant nucleic acid, as noted herein. Nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

[0041] The term "polypeptide" is used interchangeably herein with the terms "polypeptides" and "protein(s)", and refers to a polymer of amino acid residues, e.g., as typically found in proteins in nature. A "mature protein" is a protein which is full-length and which, optionally, includes glycosylation or other modifications typical for the protein in a given cell membrane.

[0042] The term "modulate" with respect to an hT1R1, hT1R2, and/or hT1R3 proteins refers to a change in the activity of hT1R1, hT1R2, and/or hT1R3 proteins. For example, modulation may cause an increase or a decrease in protein activity (e.g., coupled GTPase activity), binding characteristics, membrane permeability or any other biological, functional, or immunological properties of such proteins. The change in activity can arise from, for example, an increase or decrease in expression of one or more genes that encode these proteins, the stability of an mRNA that encodes the protein, translation efficiency, or

from a change in activity of the protein itself. For example, a molecule that binds to one of the receptors can cause an increase or decrease in the biological activity of the receptor.

[0043] The term "variant" with respect to a polypeptide refers to an amino acid sequence that is altered by one or more amino acids with respect to a reference sequence. The variant can have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. Alternatively, a variant can have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variation can also include amino acid deletion or insertion, or both. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without eliminating biological or immunological activity can be found using computer programs well known in the art, for example, DNASTAR software. Examples of conservative substitutions are also described below.

[0044] As used herein, an "antibody" is a protein comprising one or more polypeptides substantially or partially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A typical immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chains respectively. Antibodies exist as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab')2 dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (see, Fundamental Immunology, W.E. Paul,

ed., Raven Press, N.Y. (1999), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein, includes antibodies or fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies. Antibodies include multiple or single chain antibodies, including single chain Fv (sFv) antibodies in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide.

A variety of additional terms are defined or otherwise characterized herein.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0045] The present invention relates to the identification of three novel members of human sweet receptors referred to as hT1R1, hT1R2 and hT1R3 nucleic acids encoding these proteins, vectors and host cells comprising the nucleic acids, methods for producing the proteins and methods for identifying compounds which bind to and/or modulate the activity of these proteins. These genes are specifically expressed in specialized neuroepithelial cells referred to as human taste receptor cells located in the fungiform papillae of the tongue.

[0046] Evidence supports the assignment of hT1R1, hT1R2 and hT1R3 as belonging to the sweet receptor family. The hT1R1, hT1R2 and hT1R3 proteins show homology to their mouse (mT1R1, mT1R2, mT1R3) and rat (rT1R1, rT1R2) counterparts (*see*, Example 1). All three hT1Rs are predicted to contain seven-transmembrane domains (*see*, Figure 1) consistent with previous studies implicating G proteins and their respective GPCRs in sweet taste transduction. In addition, all three ht1Rs are predicted to have long N-terminal extracellular domains which is characteristic of other members of the GPCR subfamily 3, which includes metabotropic glutamate receptors (as described below, the receptors herein can form heteromers that provide glutamate receptor activity as well as sweet receptor activity), extracellular Ca^{++} sensors and pheromone receptors. Chromosome mapping studies using the NCBI human genome search interface (*see*, Example 2) have demonstrated that these three genes are clustered in a region of human chromosome 1. The

aforementioned region of the human chromosome is syntenous to the distal end of mouse chromosome 4 which contains the *Sac* locus, which in turn has been implicated in detecting sweet tastants as described, e.g., in Fuller, *J. Hered.* 65:33-36, 1974; Lush et al., *Genet. Res.* 66:167-174; and Bachmanov, *Mamm. Genome* 8:545-548. *In situ* hybridization studies (*see*, Example 3) have also confirmed that these genes are specifically expressed in human taste receptor cells in the fungiform papillae of the human tongue which is consistent with their role in taste perception. Based on the amino acid homology between the hT1R proteins and their mouse and rat counterparts, the hT1R genes' expression in the fungiform papillae of the human tongue, and the location of the hT1R proteins on human chromosome 1, a syntenic region of the distal end of mouse chromosome 4 in which the mouse Sac locus maps, it is reasonable to conclude that the new hT1R proteins function as sweet receptors or receptor components. As noted below, various heteromeric versions of the proteins have been shown to respond to glutamate, implicating them as glutamate receptors as well.

[0047] Since the aforementioned genes are expressed in taste cells, these genes and their related polypeptides can serve as specific targets for the identification of sweet tastants and the design of novel sweeteners. Accordingly, the invention also relates to methods for screening compounds that bind to and/or modulate the activity of these receptors, to identify compounds that stimulate sweet taste perception.

MAKING COMPOSITIONS OF THE INVENTION

[0048] In practicing the present invention, many conventional techniques in molecular biology, microbiology, and recombinant DNA are optioanlly used. These techniques are well known and are explained in, for example, *Current Protocols in Molecular Biology*, Volumes I, II, and III, 1997 (F. M. Ausubel ed.); Sambrook et al., 2001, *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *DNA Cloning: A Practical Approach*, Volumes I and II, 1985 (D. N. Glover ed.); *Oligonucleotide Synthesis*, 1984 (M. L. Gait ed.); *Nucleic Acid Hybridization*, 1985, (Hames and Higgins); *Transcription and Translation*, 1984 (Hames and Higgins eds.); *Animal Cell Culture*, 1986 (R. I. Freshney ed.); *Immobilized Cells and Enzymes*, 1986 (IRL Press); Perbal, 1984, *A Practical Guide to Molecular Cloning*; the series, *Methods in Enzymology* (Academic Press, Inc.); *Gene Transfer Vectors for*

Mammalian Cells, 1987 (J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory); and *Methods in Enzymology* Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively).

Nucleic Acids

[0049] In one aspect, the invention provides isolated nucleic acids encoding a hT1R1 protein. These include the isolated nucleic acid molecule encoding hT1R1 protein comprising an amino acid sequence as set forth in SEQ ID NO:1 (Figure 1) and the isolated nucleic acid molecule encoding a hT1R1 protein comprising a nucleotide sequence as set forth in SEQ ID NO:10 (Figure 2A) as well as a wide variety of variants as noted herein.

[0050] In another aspect, the invention provides isolated nucleic acids encoding a hT1R2 protein. These include the isolated nucleic acid molecule encoding hT1R2 protein comprising an amino acid sequence as set forth in SEQ ID NO:4 (Figure 1) and the isolated nucleic acid molecule encoding a hT1R2 protein comprising a nucleotide sequence as set forth in SEQ ID NO:11 (Figure 2B) as well as a wide variety of variants as noted herein.

[0051] In another aspect, the invention provides isolated nucleic acids encoding a hT1R3 protein. These include the isolated nucleic acid molecule encoding hT1R3 protein comprising an amino acid sequence as set forth in SEQ ID NO:7 (Figure 1) and the isolated nucleic acid molecule encoding a hT1R3 protein comprising a nucleotide sequence as set forth in SEQ ID NO:12 (Figure 2C) as well as a wide variety of variants as noted herein.

[0052] Nucleic acid molecules of the present invention also include isolated nucleic acid molecules that have at least 50% identity or more, typically at least 60% identity or more, generally 70% identity or more, often 80% identity or more, e.g., 90% identity or more, preferably at least 95% identity, more preferably at least 98% identity, and most preferably at least 99% identity to a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:1, SEQ ID NO:4 and/or SEQ ID NO:7, respectively. Such nucleic acid molecules include a nucleic acid encoding a polypeptide of SEQ ID NO:1, SEQ ID NO:4 and SEQ ID NO:7 as set forth above. The identity can be over the entire coding region, or can be over a subsequence, e.g., a subsequence comprising at least about 10%, e.g., at least 25%, e.g., at least 50% or more of the full-length sequence. Nucleic acids

of the present invention also include fragments of the aforementioned nucleic acid molecules. For example, the invention provides nucleic acids that encode one or more of the domains of the hT1R receptors. Such domains include the amino terminal extracellular domain, the seven transmembrane (TM) domains, the extracellular domains (located between TM2 and TM3, between TM4 and TM5, and between TM6 and TM7), and the intracellular domains (C-terminal to TM7, and between TM1 and TM2, between TM3 and TM4, and between TM5 and TM6). The amino acid sequences of the transmembrane domains, intracellular domains, and extracellular domains are shown in, for example, Figure 1.

[0053] Nucleic acids of the present invention include isolated nucleic acid molecules encoding polypeptide variants which comprise the amino acid sequences of SEQ ID NO:1, SEQ ID NO:4 and SEQ ID NO:7 (h1R1, h1R2 and h1R3, respectively). Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

[0054] The invention also provides isolated nucleic acid molecules that are fully complementary to all the above described isolated nucleic acid molecules.

[0055] An isolated nucleic acid encoding one of the above polypeptides including homologs from species other than rat, mouse or human, may be obtained by a method which comprises the steps of screening an appropriate library under stringent conditions with a labeled probe having the sequence of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, or a fragment thereof; and isolating cDNA and genomic clones containing the nucleotide sequences. Such hybridization techniques are well-known to a skilled artisan. Another typical method for making appropriate sequences includes performing PCR on genomic or cDNA from an appropriate library or nucleic acid preparation.

[0056] Nucleic acid molecules encoding the above hT1R receptors and variants thereof can be obtained from genomic or cDNA, can be amplified via PCR or LCR, or can be synthesized, or made by any combination of conventional techniques. The DNA can then be used to express the hT1R protein, or as a template for preparation of RNA or as a molecular probe which selectively hybridizes to, and thus can detect the presence of, other T1Rx-encoding nucleotide sequences. Naturally occurring sequences can be mutated, e.g., by point mutagenesis or DNA shuffling or other available mutagenesis methods to make

variants that are within the scope of the invention. One of skill will also appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. *See,* Ausubel, Sambrook and Berger, *herein.* In addition, RNAs of the invention can be made by transcription of DNA sequences.

[0057] When nucleic acid molecules of the present invention are utilized for the recombinant production of hT1R polypeptides of the present invention, the nucleotide sequence can include the coding sequence for the mature polypeptide, by itself; or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded, e.g., a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., *Proc. Nat'l. Acad. Sci. USA* (1989) 86:821-824, or is an HA tag. The nucleic acid molecule can also contain noncoding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

[0058] General texts which describe molecular biological techniques for making nucleic acids, including the use of vectors, promoters and many other relevant topics, include Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA (Berger); Sambrook et al., Molecular Cloning - A Laboratory Manual (3nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2000 ("Sambrook") and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2002) ("Ausubel")). Examples of techniques sufficient to direct persons of skill through *in vitro* amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques (*e.g.,* NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis *et al.*, (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) *C&EN*

36-47; *The Journal Of NIH Research* (1991) 3, 81-94; (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86, 1173; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87, 1874; Lomell *et al.* (1989) *J. Clin. Chem* 35, 1826; Landegren *et al.*, (1988) *Science* 241, 1077-1080; Van Brunt (1990) *Biotechnology* 8, 291-294; Wu and Wallace, (1989) *Gene* 4, 560; Barringer *et al.* (1990) *Gene* 89, 117, and Sooknanan and Malek (1995) *Biotechnology* 13: 563-564.

[0059] In addition, a plethora of kits are commercially available for the purification of plasmids or other relevant nucleic acids from cells, (*see*, e.g., EasyPrep™, FlexiPrep™, both from Pharmacia Biotech; StrataClean™, from Stratagene; and, QIAprep™ from Qiagen). Any isolated and/or purified nucleic acid can be further manipulated to produce other nucleic acids, used to transfect cells, incorporated into related vectors to infect organisms, or the like. Typical cloning vectors contain transcription and translation terminators, transcription and translation initiation sequences, and promoters useful for regulation of the expression of the particular target nucleic acid. The vectors optionally comprise generic expression cassettes containing at least one independent terminator sequence, sequences permitting replication of the cassette in eukaryotes, or prokaryotes, or both, (e.g., shuttle vectors) and selection markers for both prokaryotic and eukaryotic systems. Vectors are suitable for replication and integration in prokaryotes, eukaryotes, or both. *See,* Giliman & Smith, *Gene* 8:81 (1979); Roberts, *et al.*, *Nature*, 328:731 (1987); Schneider, B., *et al.*, *Protein Expr. Purif.* 6435:10 (1995); Ausubel, Sambrook, Berger (*above*). A catalogue of Bacteria and Bacteriophages useful for cloning is provided, e.g., by the ATCC, e.g., *The ATCC Catalogue of Bacteria and Bacteriophage* published yearly by the ATCC. Additional basic procedures for sequencing, cloning and other aspects of molecular biology and underlying theoretical considerations are also found in Watson *et al.* (1992) *Recombinant DNA Second Edition, Scientific American Books, NY.*

[0060] Other useful references, e.g. for cell isolation and culture (e.g., for subsequent nucleic acid isolation) include Freshney (1994) Culture of Animal Cells, a Manual of Basic Technique, third edition, Wiley- Liss, New York and the references cited therein; Payne *et al.* (1992) Plant Cell and Tissue Culture in Liquid Systems John Wiley & Sons, Inc. New York, NY; Gamborg and Phillips (eds) (1995) Plant Cell, Tissue and Organ Culture; Fundamental Methods Springer Lab Manual, Springer-Verlag (Berlin Heidelberg

New York) and Atlas and Parks (eds) The Handbook of Microbiological Media (1993) CRC Press, Boca Raton, FL.

[0061] In addition, essentially any nucleic acid (and virtually any labeled nucleic acid, whether standard or non-standard) can be custom or standard ordered from any of a variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com), The Great American Gene Company (www.genco.com), ExpressGen Inc. (www.expressgen.com), Operon Technologies Inc. (Alameda, CA) and many others.

[0062] Various types of mutagenesis are optionally used in the present invention, e.g., to modify hT1R1, hT1R2 or hT1R3 nucleic acids and encoded polypeptides to produce conservative or non-conservative variants. Any available mutagenesis procedure can be used. Such mutagenesis procedures optionally include selection of mutant nucleic acids and polypeptides for one or more activity of interest. Procedures that can be used include, but are not limited to: site-directed point mutatgenesis, random point mutagenesis, in vitro or in vivo homologous recombination (DNA shuffling), mutagenesis using uracil containing templates, oligonucleotide-directed mutagenesis, phosphorothioate-modified DNA mutagenesis, mutagenesis using gapped duplex DNA, point mismatch repair, mutagenesis using repair-deficient host strains, restriction-selection and restriction-purification, deletion mutagenesis, mutagenesis by total gene synthesis, double-strand break repair, and many others known to persons of skill. Mutagenesis, e.g., involving chimeric constructs, are also included in the present invention. In one embodiment, mutagenesis can be guided by known information of the naturally occurring molecule or altered or mutated naturally occurring molecule, e.g., sequence, sequence comparisons, physical properties, crystal structure or the like. In another class of embodiments, modification is essentially random (e.g., as in classical DNA shuffling).

[0063] The above texts describe these procedures. Additional information is found in the following publications and references cited within: Arnold, *Protein engineering for unusual environments*, Current Opinion in Biotechnology 4:450-455 (1993); Bass et al., *Mutant Trp repressors with new DNA-binding specificities*, Science 242:240-245 (1988); Botstein & Shortle, *Strategies and applications of in vitro mutagenesis*, Science 229:1193-1201(1985); Carter et al., *Improved oligonucleotide site-directed mutagenesis using M13 vectors*, Nucl. Acids Res. 13: 4431-4443 (1985); Carter, *Site-directed mutagenesis*,

Biochem. J. 237:1-7 (1986); Carter, *Improved oligonucleotide-directed mutagenesis using M13 vectors*, Methods in Enzymol. 154: 382-403 (1987); Dale et al., *Oligonucleotide-directed random mutagenesis using the phosphorothioate method*, Methods Mol. Biol. 57:369-374 (1996); Eghtedarzadeh & Henikoff, *Use of oligonucleotides to generate large deletions*, Nucl. Acids Res. 14: 5115 (1986); Fritz et al., *Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro*, Nucl. Acids Res. 16: 6987-6999 (1988); Grundström et al., *Oligonucleotide-directed mutagenesis by microscale 'shot-gun' gene synthesis*, Nucl. Acids Res. 13: 3305-3316 (1985); Kunkel, *The efficiency of oligonucleotide directed mutagenesis*, in Nucleic Acids & Molecular Biology (Eckstein, F. and Lilley, D.M.J. eds., Springer Verlag, Berlin)) (1987); Kunkel, *Rapid and efficient site-specific mutagenesis without phenotypic selection*, Proc. Natl. Acad. Sci. USA 82:488-492 (1985); Kunkel et al., *Rapid and efficient site-specific mutagenesis without phenotypic selection*, Methods in Enzymol. 154, 367-382 (1987); Kramer et al., *The gapped duplex DNA approach to oligonucleotide-directed mutation construction*, Nucl. Acids Res. 12: 9441-9456 (1984); Kramer & Fritz *Oligonucleotide-directed construction of mutations via gapped duplex DNA*, Methods in Enzymol. 154:350-367 (1987); Kramer et al., *Point Mismatch Repair*, Cell 38:879-887 (1984); Kramer et al., *Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations*, Nucl. Acids Res. 16: 7207 (1988); Ling et al., *Approaches to DNA mutagenesis: an overview*, Anal Biochem. 254(2): 157-178 (1997); Lorimer and Pastan Nucleic Acids Res. 23, 3067-8 (1995); Mandecki, *Oligonucleotide-directed double-strand break repair in plasmids of Escherichia coli: a method for site-specific mutagenesis*, Proc. Natl. Acad. Sci. USA, 83:7177-7181 (1986); Nakamaye & Eckstein, *Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis*, Nucl. Acids Res. 14: 9679-9698 (1986); Nambiar et al., *Total synthesis and cloning of a gene coding for the ribonuclease S protein*, Science 223: 1299-1301 (1984); Sakamar and Khorana, *Total synthesis and expression of a gene for the a-subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin)*, Nucl. Acids Res. 14: 6361-6372 (1988); Sayers et al., *Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis*, Nucl. Acids Res. 16:791-802 (1988); Sayers et al., *Strand specific cleavage of*

phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide, (1988) Nucl. Acids Res. 16: 803-814; Sieber, et al., Nature Biotechnology, 19:456-460 (2001); Smith, *In vitro mutagenesis*, Ann. Rev. Genet. 19:423-462(1985); Methods in Enzymol. 100: 468-500 (1983); Methods in Enzymol. 154: 329-350 (1987); Stemmer, Nature 370, 389-91 (1994); Taylor et al., *The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA*, Nucl. Acids Res. 13: 8749-8764 (1985); Taylor et al., *The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA*, Nucl. Acids Res. 13: 8765-8787 (1985); Wells et al., *Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin*, Phil. Trans. R. Soc. Lond. A 317: 415-423 (1986); Wells et al., *Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites*, Gene 34:315-323 (1985); Zoller & Smith, *Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment*, Nucleic Acids Res. 10:6487-6500 (1982); Zoller & Smith, *Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors*, Methods in Enzymol. 100:468-500 (1983); and Zoller & Smith, *Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template*, Methods in Enzymol. 154:329-350 (1987). Additional details on many of the above methods can be found in Methods in Enzymology Volume 154, which also describes useful controls for trouble-shooting problems with various mutagenesis methods.

Polypeptides

[0064] In another aspect, the present invention relates to hT1R polypeptides. These include the hT1R1 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:1 (Figure 1), the hT1R2 polypeptide comprising an amino acid sequence as set forth in SEQ ID:4 (Figure 1) and the hT1R3 polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:7 (Figure 1) as well as variants thereof. The polypeptides of the present invention also include fragments of the aforementioned sequences. For example, the invention also provides polypeptides that comprise one or more domains of the hT1R receptor polypeptides. These domains, which include extracellular domains, intracellular domains, and transmembrane domains, are described above and shown in Figure 1.

[0065] Polypeptides of the present invention include isolated polypeptides, e.g., variants, in which the amino acid sequence has at least 75% identity, preferably at least 80% identity, typically 90% identity, preferably at least 95% identity, more preferably at least 98% identity and most preferably at least 99% identity, to the amino acid sequences as set forth in SEQ ID NO:1, SEQ ID NO:4 and SEQ ID NO:7. Such sequences include the sequences of SEQ ID NO:1, SEQ ID NO:4 and SEQ ID NO:7 as set forth above.

[0066] The aforementioned hT1R polypeptides can be obtained by any of a variety of methods. Smaller peptides (less than 50 amino acids long) are conveniently synthesized by standard chemical techniques and can be chemically or enzymatically ligated to form larger polypeptides. Polypeptides can be purified from biological sources by methods well known in the art (*see*, e.g., Protein Purification, Principles and Practice, Second Edition (1987) Scopes, Springer Verlag, N.Y.). They are optionally (and preferably) produced in their naturally occurring, truncated, or fusion protein forms by recombinant DNA technology using techniques well known in the art. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook et al. (2001) *Molecular Cloning, A Laboratory Manual*, Third Edition, Cold Spring Harbor Press, N.Y.; and Ausubel et al., eds. (1997) *Current Protocols in Molecular Biology*, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., N.Y (supplemented through 2002). Alternatively, RNA encoding the proteins can be chemically synthesized. See, for example, the techniques described in *Oligonucleotide Synthesis*, (1984) Gait ed., IRL Press, Oxford, which is incorporated by reference herein in its entirety. Obtaining large quantities of these polypeptides is preferably by recombinant techniques as further described above under the section entitled "making nucleic acids."

[0067] Another aspect of the present invention relates to a method for producing a hT1R1, ht1R2 or hT1R3 polypeptide, or a polypeptide that comprises one or more domains thereof. These methods involve, e.g.,:

a) culturing a host cell comprising a nucleic acid of the invention, e.g., a nucleic acid encoding an hT1R1, hT1R2 or hT1R3 polypeptide, or variant or domain thereof, under conditions suitable for expression of the hT1R1, hT1R2 or hT1R3 polypeptide; and

b) isolating the hT1R1, hT1R2 or hT1R3 polypeptide or domain thereof.

[0068] As described, the nucleic acid molecules described herein can be expressed in a suitable host cell to produce active hT1R1, hT1R2 or hT1R3 protein. Expression occurs by placing a nucleotide sequence encoding these proteins into an appropriate expression vector and introducing the expression vector into a suitable host cell, culturing the transformed host cell under conditions suitable for expression of the hT1R1, hT1R2, hT1R3 protein or variant thereof, or a polypeptide that comprises one or more domains of such proteins, and purifying the recombinant proteins from the host cell to obtain purified, and preferably active, hT1R1, hT1R2 or hT1R3 protein. Appropriate expression vectors are known in the art. For example, pET-14b, pCDNA1Amp, and pVL1392 are available from Novagen and Invitrogen and are suitable vectors for expression in *E. coli*, COS cells and baculovirus infected insect cells, respectively. These vectors are illustrative of those that are known in the art. Suitable host cells can be any cell capable of growth in a suitable media and allowing purification of the expressed protein. Examples of suitable host cells include bacterial cells, such as *E. coli*, *Streptococci*, *Staphylococci*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells such as yeast cells, e.g., *Pichia*, and *Aspergillus* cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells, mammalian cells such as CHO, COS, HeLa; and plant cells.

[0069] Culturing and growth of the transformed host cells can occur under conditions that are known in the art. The conditions will generally depend upon the host cell and the type of vector used. Suitable culturing conditions may be used such as temperature and chemicals and will depend on the type of promoter utilized. In addition to Sambrook, Berger, Ausubel and the other references previously noted, details regarding cell culture can also be found in Freshney (1994) *Culture of Animal Cells, a Manual of Basic Technique*, third edition, Wiley- Liss, New York Payne *et al.* (1992) *Plant Cell and Tissue Culture in Liquid Systems* John Wiley & Sons, Inc. New York, NY; Gamborg and Phillips (eds) (1995) *Plant Cell, Tissue and Organ Culture*; *Fundamental Methods* Springer Lab Manual, Springer-Verlag (Berlin Heidelberg New York); and Atlas and Parks (eds) *The Handbook of Microbiological Media* (1993) CRC Press, Boca Raton, FL.

[0070] Purification of the hT1R1, hT1R2 or hT1R3 protein, or domains of such proteins, can be accomplished using known techniques without performing undue experimentation. Generally, the transformed cells expressing one of these proteins are

broken, crude purification occurs to remove debris and some contaminating proteins, followed by chromatography to further purify the protein to the desired level of purity. Cells can be broken by known techniques such as homogenization, sonication, detergent lysis and freeze-thaw techniques. Crude purification can occur using ammonium sulfate precipitation, centrifugation or other known techniques. Suitable chromatography includes anion exchange, cation exchange, high performance liquid chromatography (HPLC), gel filtration, affinity chromatography, hydrophobic interaction chromatography, etc. Well known techniques for refolding proteins can be used to obtain the active conformation of the protein when the protein is denatured during intracellular synthesis, isolation or purification.

[0071] In general, proteins of the invention, e.g., proteins comprising hT1R1, hT1R2 and/or hT1R3 sequences or domains, or antibodies to such proteins can be purified, either partially (e.g., achieving a 5X, 10X, 100X, 500X, or 1000X or greater purification), or even substantially to homogeneity (e.g., where the protein is the main component of a solution, typically excluding the solvent (e.g., water or DMSO) and buffer components (e.g., salts and stabilizers) that the protein is suspended in, e.g., if the protein is in a liquid phase), according to standard procedures known to and used by those of skill in the art. Accordingly, polypeptides of the invention can be recovered and purified by any of a number of methods well known in the art, including, e.g., ammonium sulfate or ethanol precipitation, acid or base extraction, column chromatography, affinity column chromatography, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography, lectin chromatography, gel electrophoresis and the like. Protein refolding steps can be used, as desired, in making correctly folded mature proteins. High performance liquid chromatography (HPLC), affinity chromatography or other suitable methods can be employed in final purification steps where high purity is desired. In one embodiment, antibodies made against hT1R1, hT1R2 and/or hT1R3 (or proteins comprising hT1R1, hT1R2 and/or hT1R3 domains) are used as purification reagents, e.g., for affinity-based purification of proteins comprising one or more hT1R1, hT1R2 and/or hT1R3 domains or antibodies thereto. Once purified, partially or to homogeneity, as desired, the polypeptides are optionally used e.g., as assay components, therapeutic reagents or as immunogens for antibody production.

[0072] In addition to other references noted herein, a variety of purification/protein purification methods are well known in the art, including, e.g., those set forth in R. Scopes, Protein Purification, Springer-Verlag, N.Y. (1982); Deutscher, Methods in Enzymology Vol. 182: Guide to Protein Purification, Academic Press, Inc. N.Y. (1990); Sandana (1997) Bioseparation of Proteins, Academic Press, Inc.; Bollag et al. (1996) Protein Methods, 2nd Edition Wiley-Liss, NY; Walker (1996) The Protein Protocols Handbook Humana Press, NJ; Harris and Angal (1990) Protein Purification Applications: A Practical Approach IRL Press at Oxford, Oxford, England; Harris and Angal Protein Purification Methods: A Practical Approach IRL Press at Oxford, Oxford, England; Scopes (1993) Protein Purification: Principles and Practice 3rd Edition Springer Verlag, NY; Janson and Ryden (1998) Protein Purification: Principles, High Resolution Methods and Applications, Second Edition Wiley-VCH, NY; and Walker (1998) Protein Protocols on CD-ROM Humana Press, NJ; and the references cited therein.

[0073] Those of skill in the art will recognize that, after synthesis, expression and/or purification, proteins can possess a conformation different from the desired conformations of the relevant polypeptides. For example, polypeptides produced by prokaryotic systems often are optimized by exposure to chaotropic agents to achieve proper folding. During purification from, e.g., lysates derived from E. coli, the expressed protein is optionally denatured and then renatured. This is accomplished, e.g., by solubilizing the proteins in a chaotropic agent such as guanidine HCl. In general, it is occasionally desirable to denature and reduce expressed polypeptides and then to cause the polypeptides to re-fold into the preferred conformation. For example, guanidine, urea, DTT, DTE, and/or a chaperonin can be added to a translation product of interest. Methods of reducing, denaturing and renaturing proteins are well known to those of skill in the art (see, the references above, and Debinski, et al. (1993) J. Biol. Chem., 268: 14065-14070; Kreitman and Pastan (1993) Bioconjug. Chem.,4: 581-585; and Buchner, et al., (1992) Anal. Biochem., 205: 263-270). Debinski, et al., for example, describe the denaturation and reduction of inclusion body proteins in guanidine-DTE. The proteins can be refolded in a redox buffer containing, e.g., oxidized glutathione and L-arginine. Refolding reagents can be flowed or otherwise moved into contact with the one or more polypeptide or other expression product, or vice-versa.

[0074] The polynucleotides of the present invention optionally comprise a coding sequence fused in-frame to a marker sequence which, *e.g.*, facilitates purification of the encoded polypeptide. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, a sequence which binds glutathione (*e.g.*, GST), a hemagglutinin (HA) tag (corresponding to an epitope derived from the influenza hemagglutinin protein; Wilson, I., *et al.* (1984) *Cell* 37:767), maltose binding protein sequences, the FLAG epitope utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA), and the like. The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and the sequence of the invention is useful to facilitate purification.

Sequence Variations
Silent Variations

[0075] Due to the degeneracy of the genetic code, any of a variety of nucleic acids sequences encoding polypeptides of the invention are optionally produced, some which can bear lower levels of sequence identity to the hT1Rx nucleic acid and polypeptide sequences in the figures. The following provides a typical codon table specifying the genetic code, found in many biology and biochemistry texts.

Table 1
Codon Table

Amino acids			Codon					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

[0076] The codon table shows that many amino acids are encoded by more than one codon. For example, the codons AGA, AGG, CGA, CGC, CGG, and CGU all encode the amino acid arginine. Thus, at every position in the nucleic acids of the invention where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described above without altering the encoded polypeptide. It is understood that U in an RNA sequence corresponds to T in a DNA sequence.

[0077] Using, as an example, the nucleic acid sequence corresponding to nucleotides 1-18 of SEQ ID NO: 10 are: ATG CTG CTC TGC ACG GCT (MLLCTA from SEQ ID NO: 1). A silent variation of this sequence includes ATG, TTA TTG TGT, ACC, GCC (also encoding MLLCTA from SEQ ID NO:1).

[0078] Such "silent variations" are one species of "conservatively modified variations", discussed below. One of skill will recognize that each codon in a nucleic acid (except ATG, which is ordinarily the only codon for methionine) can be modified by standard techniques to encode a functionally identical polypeptide. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in any described

sequence. The invention, therefore, explicitly provides each and every possible variation of a nucleic acid sequence encoding a polypeptide of the invention that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code (e.g., as set forth in Table 1, or as is commonly available in the art) as applied to the nucleic acid sequence encoding a T1Rx polypeptide of the invention. All such variations of every nucleic acid herein are specifically provided and described by consideration of the sequence in combination with the genetic code. One of skill is fully able to make these silent substitutions using the methods herein.

Conservative Variations

[0079] "Conservatively modified variations" or, simply, "conservative variations" of a particular nucleic acid sequence or polypeptide are those which encode identical or essentially identical amino acid sequences. One of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 4%, 2% or 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the deletion of an amino acid, addition of an amino acid, or substitution of an amino acid with a chemically similar amino acid.

[0080] Conservative substitution tables providing functionally similar amino acids are well known in the art. Table 2 sets forth six groups which contain amino acids that are "conservative substitutions" for one another.

Table 2

Conservative Substitution Groups

1	Alanine (A)	Serine (S)	Threonine (T)	
2	Aspartic acid (D)	Glutamic acid (E)		
3	Asparagine (N)	Glutamine (Q)		
4	Arginine (R)	Lysine (K)		
5	Isoleucine (I)	Leucine (L)	Methionine (M)	Valine (V)
6	Phenylalanine (F)	Tyrosine (Y)	Tryptophan (W)	

[0081] Thus, "conservatively substituted variations" of a listed polypeptide sequence of the present invention include substitutions of a small percentage, typically less

than 5%, more typically less than 2% or 1%, of the amino acids of the polypeptide sequence, with a conservatively selected amino acid of the same conservative substitution group.

[0082] For example, a conservatively substituted variation of the polypeptide identified herein as SEQ ID NO:1 will contain "conservative substitutions", according to the six groups defined above, in up to about 40 residues (i.e., about 5% of the amino acids) in the full-length polypeptide.

[0083] In a further example, if conservative substitutions were localized in the region corresponding to amino acids 5-10 (TARLV), examples of conservatively substituted variations of this region include conservative exchange of conserved amino acids, e.g., substitution of STKMM or TSKVI (or any others that can be made according to Table 2) for TARLV. Listing of a protein sequence herein, in conjunction with the above substitution table, provides an express listing of all conservatively substituted proteins.

[0084] Finally, the addition or deletion of sequences which do not alter the encoded activity of a nucleic acid molecule, such as the addition or deletion of a non-functional sequence, is a conservative variation of the basic nucleic acid or polypeptide.

[0085] One of skill will appreciate that many conservative variations of the nucleic acid constructs which are disclosed yield a functionally identical construct. For example, as discussed above, owing to the degeneracy of the genetic code, "silent substitutions" (*i.e.,* substitutions in a nucleic acid sequence which do not result in an alteration in an encoded polypeptide) are an implied feature of *every* nucleic acid sequence which encodes an amino acid. Similarly, "conservative amino acid substitutions," in one or a few amino acids in an amino acid sequence are substituted with different amino acids with highly similar properties, are also readily identified as being highly similar to a disclosed construct. Such conservative variations of each disclosed sequence are a feature of the present invention.

hT1R1, hT1R2 and/or hT1R3 Antibodies

[0086] In another aspect, antibodies to hT1R1, hT1R2 or hT1R3 proteins or fragments thereof can be generated using methods that are well known in the art. The antibodies can be utilized for detecting and/or purifying the hT1Rx proteins, optionally discriminating the proteins from various homologues, and/or in biosensor hT1R1, hT1R2 or hT1R3 activity detection applications. As used herein, the term antibody includes, but is not

limited to, polyclonal antibodies, monoclonal antibodies, humanized or chimeric antibodies and biologically functional antibody fragments, which are those fragments sufficient for binding of the antibody fragment to the protein.

[0087] For the production of antibodies to a protein encoded by one of the disclosed genes, various host animals may be immunized by injection with the polypeptide, or a portion thereof. Such host animals may include, but are not limited to, rabbits, mice and rats, to name but a few. Various adjuvants may be used to enhance the immunological response, depending on the host species, including, but not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum.*

[0088] Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals, such as those described above, may be immunized by injection with the encoded protein, or a portion thereof, supplemented with adjuvants as also described above.

[0089] Monoclonal antibodies (mAbs), which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein (*Nature* 256:495-497, 1975; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Nat'l. Acad. Sci. USA* 80:2026-2030, 1983), and the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96, 1985). Such antibodies may be of any immunoglobulin class, including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo.* Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

[0090] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Nat'l. Acad. Sci. USA* 81:6851-6855, 1984; Neuberger et al., *Nature* 312:604-608, 1984; Takeda et al., *Nature* 314:452-454, 1985) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity, together with genes from a human antibody molecule of appropriate biological activity, can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable or hypervariable region derived from a murine mAb and a human immunoglobulin constant region.

[0091] Alternatively, techniques described for the production of single-chain antibodies (U.S. Patent No. 4,946,778; Bird, *Science* 242:423-426, 1988; Huston et al., *Proc. Nat'l. Acad. Sci. USA* 85:5879-5883, 1988; and Ward et al., *Nature* 334:544-546, 1989) can be adapted to produce differentially expressed gene-single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single-chain polypeptide.

[0092] In one aspect, techniques useful for the production of "humanized antibodies" can be adapted to produce antibodies to the proteins, fragments or derivatives thereof. Such techniques are disclosed in U.S. Patent Nos. 5,932,448; 5,693,762; 5,693,761; 5,585,089; 5,530,101; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,661,016; and 5,770,429.

[0093] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments, which can be produced by pepsin digestion of the antibody molecule, and the Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., Science 246:1275-1281, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

[0094] The protocols for detecting and measuring the expression of the described hT1R proteins using the above mentioned antibodies are well known in the art. Such methods include, but are not limited to, dot blotting, western blotting, competitive and noncompetitive protein binding assays, enzyme-linked immunosorbant assays (ELISA), immunohistochemistry, fluorescence-activated cell sorting (FACS), and others commonly

used and widely described in scientific and patent literature, and many employed commercially.

[0095] Particularly preferred, for ease of detection, is the sandwich ELISA, of which a number of variations exist, all of which are intended to be encompassed by the present invention. For example, in a typical forward assay, unlabeled antibody is immobilized on a solid substrate and the sample to be tested is brought into contact with the bound molecule and incubated for a period of time sufficient to allow formation of an antibody-antigen binary complex. At this point, a second antibody, labeled with a reporter molecule capable of inducing a detectable signal, is then added and incubated, allowing time sufficient for the formation of a ternary complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal, or may be quantitated by comparing with a control sample containing known amounts of antigen. Variations on the forward assay include the simultaneous assay, in which both sample and antibody are added simultaneously to the bound antibody, or a reverse assay, in which the labeled antibody and sample to be tested are first combined, incubated and added to the unlabeled surface bound antibody. These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-site technique. For the immunoassays of the present invention, the only limiting factor is that the labeled antibody be an antibody which is specific for the protein expressed by the gene of interest.

[0096] The most commonly used reporter molecules in this type of assay are either enzymes, fluorophore- or radionuclide-containing molecules. In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, usually by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different ligation techniques exist which are well-known to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, among others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine or

toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product, rather than the chromogenic substrates noted above. A solution containing the appropriate substrate is then added to the tertiary complex. The substrate reacts with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an evaluation of the amount of PLAB which is present in the serum sample.

[0097] Alternately, fluorescent compounds, such as fluorescein and rhodamine, can be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic longer wavelength. The emission appears as a characteristic color visually detectable with a light microscope. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotopes, chemiluminescent or bioluminescent molecules may also be employed. It will be readily apparent to the skilled artisan how to vary the procedure to suit the required use.

Defining Proteins and Nucleic Acids by Immunoreactivity

[0098] Because the polypeptides of the invention provide a variety of new polypeptide sequences, the polypeptides also provide new structural features which can be recognized, e.g., in immunological assays. The generation of antisera which specifically bind the polypeptides of the invention, as well as the polypeptides which are bound by such antisera, are a feature of the invention.

[0099] For example, the invention includes hT1R1, hT1R2 and hT1R3 proteins that specifically bind to or that are specifically immunoreactive with an antibody or antisera generated against an immunogen comprising an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 7 (and/ or nucleic acids that encode such hT1R1, hT1R2 and hT1R3 proteins). To eliminate cross-reactivity with other homologues (e.g., the mouse and rat homologues), the antibody or antisera is optionally subtracted with mT1R1, mT1R2, mT1R3, rT1R1, rT1R2, and/or rT1R3 protein(s).

[0100] In one typical format, the immunoassay uses a polyclonal antiserum which was raised against one or more polypeptide comprising one or more of the sequences

corresponding to one or more of SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 7 or a substantial subsequence thereof (i.e., at least about 30% of the full length sequence provided, or typically at least about 50%, 75% or more of the sequence). The set of potential polypeptide immunogens derived from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 7 are collectively referred to below as "the immunogenic polypeptides." The resulting antisera is optionally selected to have low cross-reactivity against the control homologues (mT1R1, mT1R2, mT1R3, rT1R1, rT1R2, and/or rT1R3, e.g., as set forth in SEQ ID NO: 2, SEQ ID NO: 3 SEQ ID NO: 5 SEQ ID NO: 6 SEQ ID NO: 8 and SEQ ID NO: 9) and any such cross-reactivity is optionally removed, e.g., by immunoabsorbtion, with one or more of the control homologues, prior to use of the polyclonal antiserum in the immunoassay.

[0101] In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example, recombinant protein can be produced in a recombinant cell. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (*see*, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity. Additional references and discussion of antibodies is also found herein and can be applied here to defining polypeptides by immunoreactivity). Alternatively, one or more synthetic or recombinant polypeptide derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

[0102] Polyclonal sera are collected and titered against the immunogenic polypeptide in an immunoassay, for example, a solid phase immunoassay with one or more of the immunogenic proteins immobilized on a solid support. Polyclonal antisera with a titer of 10^6 or greater are selected, pooled and subtracted with the control polypeptides to produce subtracted pooled titered polyclonal antisera.

[0103] The subtracted pooled titered polyclonal antisera are tested for cross reactivity against the control homologues (the mouse and or rat T1Rx protein(s)) in a comparative immunoassay. In this comparative assay, discriminatory binding conditions are

determined for the subtracted titered polyclonal antisera which result in at least about a 5-10 fold higher signal to noise ratio for binding of the titered polyclonal antisera to the immunogenic polypeptide as compared to binding to the control homologues. That is, the stringency of the binding reaction is adjusted by the addition of non-specific competitors such as albumin or non-fat dry milk, and/or by adjusting salt conditions, temperature, and/or the like. These binding conditions are used in subsequent assays for determining whether a test polypeptide (a polypeptide being compared to the immunogenic polypeptides and/ or the control polypeptides) is specifically bound by the pooled subtracted polyclonal antisera. In particular, test polypeptides which show at least a 2-5x higher signal to noise ratio than the control homologues under discriminatory binding conditions, and at least about a ½ signal to noise ratio as compared to the immunogenic polypeptide(s), clearly shares substantial structural similarity with the immunogenic polypeptide as compared to the mouse or rat homologues, and is, therefore a polypeptide of the invention.

[0104] In another example, immunoassays in the competitive binding format are used for detection of a test polypeptide. For example, as noted, cross-reacting antibodies are removed from the pooled antisera mixture by immunoabsorbtion with the control polypeptides. The immunogenic polypeptide(s) are then immobilized to a solid support which is exposed to the subtracted pooled antisera. Test proteins are added to the assay to compete for binding to the pooled subtracted antisera. The ability of the test protein(s) to compete for binding to the pooled subtracted antisera as compared to the immobilized protein(s) is compared to the ability of the immunogenic polypeptide(s) added to the assay to compete for binding (the immunogenic polypeptides compete effectively with the immobilized immunogenic polypeptides for binding to the pooled antisera). The percent cross-reactivity for the test proteins is calculated, using standard calculations.

[0105] In a parallel assay, the ability of the control proteins to compete for binding to the pooled subtracted antisera is optionally determined as compared to the ability of the immunogenic polypeptide(s) to compete for binding to the antisera. Again, the percent cross-reactivity for the control polypeptides is calculated, using standard calculations. Where the percent cross-reactivity is at least 5-10x as high for the test polypeptides as compared to the control polypeptides and or where the binding of the test

polypeptides is approximately in the range of the binding of the immunogenic polypeptides, the test polypeptides are said to specifically bind the pooled subtracted antisera.

[0106] In general, the immunoabsorbed and pooled antisera can be used in a competitive binding immunoassay as described herein to compare any test polypeptide to the immunogenic and/ or control polypeptide(s). In order to make this comparison, the immunogenic, test and control polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the subtracted antisera to, e.g., an immobilized control, test or immunogenic protein is determined using standard techniques. If the amount of the test polypeptide required for binding in the competitive assay is less than twice the amount of the immunogenic polypeptide that is required, then the test polypeptide is said to specifically bind to an antibody generated to the immunogenic protein, provided the amount is at least about 5-10x as high as for the control polypeptide.

[0107] As an additional determination of specificity, the pooled antisera is optionally fully immunosorbed with the immunogenic polypeptide(s) (rather than the control polypeptides) until little or no binding of the resulting immunogenic polypeptide subtracted pooled antisera to the immunogenic polypeptide(s) used in the immunosorbtion is detectable. This fully immunosorbed antisera is then tested for reactivity with the test polypeptide. If little or no reactivity is observed (i.e., no more than 2x the signal to noise ratio observed for binding of the fully immunosorbed antisera to the immunogenic polypeptide), then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

Methods of Use/ Biosensors

[0108] In another aspect, the present invention relates to the use of the hT1R1, hT1R2 and hT1R3 proteins and/ or coding nucleic acids in methods for identifying a compound, i.e., a sweet or umami (glutamate) tastant, that interacts/binds to the protein(s) encoded by these genes. The test compound can be natural or synthetic molecules such as proteins or fragments thereof, carbohydrates, organic or inorganic compounds and/or the like. This can be achieved, e.g., by utilizing the hT1R1, hT1R2 and hT1R3 proteins of the invention, or active fragments thereof, in cell-free or cell-based assays. A variety of formats are applicable, including measurement of second messenger effects (e.g., Ca^{2+} flux assays, cAMP assays, GTPgammaS binding assays, melanophore assays; phospholipase C assays,

beta-arrestin FRET assays, and transcriptional reporter assays, e.g., using CRE, SRE, MRE, TRE, NFAT, and/ or NFkB-response elements coupled to appropriate reporters.

[0109] In one embodiment, cell-free assays for identifying such compounds comprise a reaction mixture containing a protein encoded by one of the disclosed genes and a test compound or a library of test compounds. Accordingly, one example of a cell-free method for identifying test compounds that specifically bind to the hT1R1, ht1R2 and hT1R3 proteins comprises contacting a protein or functional fragment thereof with a test compound or library of test compounds and detecting the formation of complexes by conventional methods. In particularly useful embodiments, a library of the test compounds can be synthesized on a solid substrate, e.g., plastic pins or some other surface. The test compounds are reacted with the hT1R protein or fragment thereof and washed to elute unbound protein. Bound hT1R is then detected by methods well known in the art. Purified hT1R can also be applied directly onto plates for use in the aforementioned screening method. Antibody binding to the proteins can also be detected in this format.

[0110] Interaction between molecules can also be assessed by using real-time BIA (Biomolecular Interaction Analysis, Pharmacia Biosensor AB), which detects surface plasmon resonance, an optical phenomenon. Detection depends on changes in the mass concentration of mass macromolecules at the biospecific interface and does not require labeling of the molecules. In one useful embodiment, a library of test compounds can be immobilized on a sensor surface, e.g., a wall of a micro-flow cell. A solution containing the protein or functional fragment thereof is then continuously circulated over the sensor surface. An alteration in the resonance angle, as indicated on a signal recording, indicates the occurrence of an interaction. This technique is described in more detail in the *BIAtechnology Handbook* by Pharmacia.

[0111] In yet other useful embodiments, the hT1R protein or fragment thereof can be immobilized to facilitate separation of complexes from uncomplexed forms of the protein and automation of the assay. Complexation of the protein can be achieved in any type of vessel, e.g., microtitre plates, micro-centrifuge tubes and test tubes. In particularly preferred embodiments, the protein can be fused to another protein, e.g., glutathione-S-transferase to form a fusion protein which can be adsorbed onto a matrix, e.g., glutathione Sepharose™ beads (Sigma Chemical. St. Louis, Mo.), which are then combined with the test

compound and incubated under conditions sufficient to form complexes. Subsequently, the beads are washed to remove unbound label, and the matrix is immobilized and the radiolabel is determined.

[0112] Another method for immobilizing proteins on matrices involves utilizing biotin and streptavidin. For example, the protein can be biotinylated using biotin NHS (N-hydroxy-succinimide), using well known techniques and immobilized in the well of streptavidin-coated plates.

[0113] Cell-free assays can also be used to identify agents which specifically bind and/or modulate the activity. In one embodiment, the protein is incubated with a test compound and the catalytic activity of the protein is determined. In another embodiment, the binding affinity of the protein to a target molecule can be determined by methods known in the art.

[0114] In addition to cell-free assays such as those described above, the hT1R proteins can be utilized in cell-based assay for identifying compounds which bind to and/or modulate hT1R activity.

[0115] For example, one method for identifying compounds which bind to these proteins comprises, providing a cell that expresses one of these proteins, e.g., hT1R1, combining a test compound with the cell and measuring the formation of a complex between the test compound and the hT1R protein. The cell can be a mammalian cell, a yeast cell, bacterial cell, insect cell, a human taste cell of the fungiform papillae, or any other cell expressing the hT1R protein.

[0116] In another embodiment, human taste cells or heterologous cells expressing hT1Rs, or plasma membrane preparations of such cells, can be utilized to screen for bioactivity of test compounds or peptides. As stated above, the hT1R proteins described herein are homologous to known GPCR proteins. Accordingly, the hT1R proteins are coupled to G-proteins, which mediate signal transduction. A variety of intracellular effectors have been identified as being G-protein regulated including, but not limited to, adenyl cyclase, cyclic GMP, phospholipase C, phospholipase A2 and phosphodiesterases. G-proteins also interact with a variety of ion channels, e.g., certain voltage-sensitive Ca^{++} transients. Accordingly, the level of such second messengers produced by the aforementioned intracellular effectors, and thus activity of the hT1R receptors, can be

measured by techniques, which are well known to those skilled in the art. For example, the level of cAMP produced by activation of adenyl cyclase, can be measured by competitive assays which quantities {^{3}H}cAMP in the presence of unlabeled cAMP. The GTPase activity by G proteins can be measured, e.g., in plasma membrane preparations by measuring the hydrolysis of gamma ^{32}P GTP. Breakdown of phosphatidylinositol-4,5-bisphosphate to 1, 4,5-IP3 and diacylglycerol can be monitored by measuring the amount of diacylglycerol using thin-layer chromatography, or measuring the amount of IP3 using radiolabeling techniques or HPLC. The generation of arachidonic acid by the activation of phospholipase A2 can be readily quantitated by well-known techniques.

[0117] The search for sweet (or glutamate) substances using hT1Rx genes can also be done by cell-based assay. It is known that GPCRs induce Ca^{++} flux and other signal transduction pathways. Efflux of intracellular calcium or influx of calcium from outside the cell can be measured using conventional techniques, e.g., loading cells with a Ca^{++} sensitive fluorescent dye such as fura-2 or indol-1, and measuring any change in Ca^{++} using a fluorometer, such as Fluoskan Ascent Fluorescent Plate Reader or Flurometric Imaging Plate Reader. The signal pathways initiated by hT1Rs in response to sweet compounds can also be monitored by reporter gene assays. The co-localization of hT1R2 and hT1R3 in the same taste cell of human tongue may indicate the co-expression of hT1R2 and hT1R3 genes in the heterologous cell system is required for their activities. The co-expression of promiscuous G proteins with hT1Rs may help to funnel heterologous signal transduction of hT1Rs through a common pathway involving phospholipase C and Ca^{++} mobilization.

[0118] As described, other assays such as melanophore assays, Phospholipase C assays, beta-arrestin FRET assays, and Transcriptional reporter assays, e.g., using CRE, SRE, MRE, TRE, NFAT, and/ or NFkB-response elements coupled to appropriate reporters can be used. Detection using reporter genes coupled to appropriate response elements are particularly convenient. For example, the coding sequence to chloramphenicol acetyl transferase, beta galactosidase or other convenient markers are coupled to a response element that is activated by a second messenger that is activated by a protein of the invention. Cells expressing the marker in response to application of an appropriate test

compound are detected by cell survival, or by expression of a colorimetric marker, or the like, according to well established methods.

[0119] In an alternate embodiment, conformational changes are detected by coupling the polypeptides of the invention to an electrical readout, e.g., to a chemically coupled field effect transistor (a CHEM-FET) or other appropriate system for detecting changes in conductance or other electrical properties brought about by a conformational shift by the protein of the invention.

[0120] In an alternate aspect, potential modulators of hT1R1, hT1R2 and/or hT1R3 activity or expression can be screened for. For example, potential modulators (small molecules, organic molecules, inorganic molecules, proteins, hormones, transcription factors, or the like) can be contacted to a cell and an effect on hT1R1, hT1R2 and/or hT1R3 activity or expression (or both) can be screened for. For example, expression of hT1R1, hT1R2 and/or hT1R3 can be detected, e.g., via northern analysis or quantitative (optionally real time) RT-PCR, before and after application of potential expression modulators. Similarly, promoter regions of the various genes (e.g., generally sequences in the region of the start site of transcription, e.g., within 5 KB of the start site, e.g., 1KB, or less e.g., within 500BP or 250BP or 100 BP of the start site) can be coupled to reporter constructs (CAT, beta-galactosidase, luciferase or any other available reporter) and can be similarly be tested for expression activity modulation by the potential modulator. In either case, the assays can be performed in a high-throughput fashion, e.g., using automated fluid handling and/or detection systems, in serial or parallel fashion. Similarly, activity modulators can be tested by contacting a potential modulator to an appropriate cell using any of the activity detection methods herein, regardless of whether the activity that is detected is the result of activity modulation, expression modulation or both.

[0121] Biosensors of the invention are devices or systems that comprise the proteins of the invention coupled to a readout that measures or displays one or more activity of the protein. Thus, any of the above described assay components can be configured as a biosensor by operably coupling the appropriate assay components to a readout. The readout can be optical (e.g., to detect cell markers or cell survival) electrical (e.g., coupled to a FET, a BIAcore, or any of a variety of others), spectrographic, or the like, and can optionally include a user-viewable display (e.g., a CRT or optical viewing station). The biosensor can

be coupled to robotics or other automation, e.g., microfluidic systems, that direct contact of the test compounds to the proteins of the invention, e.g., for automated high-throughput analysis of test compound activity. A large variety of automated systems that can be adapted to use with the biosensors of the invention are commercially available. For example, automated systems have been made to assess a variety of biological phenomena, including, e.g., expression levels of genes in response to selected stimuli (Service (1998) "Microchips Arrays Put DNA on the Spot" Science 282:396-399). Laboratory systems can also perform, e.g., repetitive fluid handling operations (e.g., pipetting) for transferring material to or from reagent storage systems that comprise arrays, such as microtiter trays or other chip trays, which are used as basic container elements for a variety of automated laboratory methods. Similarly, the systems manipulate, e.g., microtiter trays and control a variety of environmental conditions such as temperature, exposure to light or air, and the like. Many such automated systems are commercially available. Examples of automated systems are available from the Zymark Corporation (Zymark Center, Hopkinton, MA), which utilize various Zymate systems (*see also*, www.zymark.com/), which typically include, e.g., robotics and fluid handling modules. Similarly, the common ORCA® robot, which is used in a variety of laboratory systems, e.g., for microtiter tray manipulation, is also commercially available, e.g., from Beckman Coulter, Inc. (Fullerton, CA). A number of automated approaches to high-throughput activity screening are provided by the Genomics Institute of the Novartis Foundation (La Jolla, CA); *See* GNF.org on the world-wide web. Microfluidic screening applications are commercially available from Caliper Technologies Corp. (Mountain View, CA). For example, (e.g., LabMicrofluidic device® high throughput screening system (HTS) by Caliper Technologies, Mountain View, CA or the HP/Agilent technologies Bioanalyzer using LabChip™ technology by Caliper Technologies Corp. can be adapted for use in the present invention.

Data Systems Comprising hT1R1, hT1R2 and hT1R3 sequences

[0122] The present invention provides databases, computers, computer readable media and systems comprising character strings corresponding to the sequence information herein for the polypeptides and nucleic acids herein, including, e.g., those sequences listed herein and the various silent substitutions and conservative substitutions thereof.

[0123] Various methods known in the art can be used to detect homology or similarity between different character strings, or can be used to perform other desirable functions such as to control output files, provide the basis for making presentations of information including the sequences and the like. Examples include BLAST, discussed *supra*.

[0124] Thus, different types of homology and similarity of various stringency and length can be detected, predicted and/or recognized in the data systems herein. For example, many homology determination methods have been designed for comparative analysis of sequences of biopolymers (nucleic acids, proteins, etc.), for spell-checking in word processing, and for data retrieval from various databases. With an understanding of hydrogen bonding between the principal nucleobases in natural polynucleotides, models that simulate annealing of complementary homologous polynucleotide strings can also be used as a foundation of sequence alignment or other operations typically performed on the character strings corresponding to the sequences herein (*e.g.*, word-processing manipulations, construction of figures comprising sequence or subsequence character strings, output tables, etc.). An example of a software package for calculating sequence similarity is BLAST, which can be adapted to the present invention by inputting character strings corresponding to the sequences herein. CLUSTAL provides another appropriate package.

[0125] Similarly, standard desktop applications such as word processing software (e.g., Microsoft Word™ or Corel WordPerfect™) and database software (e.g., spreadsheet software such as Microsoft Excel™, Corel Quattro Pro™, or database programs such as Microsoft Access™ or Sequel™, Oracle™, Paradox™) can be adapted to the present invention by inputting a character string corresponding to the proteins or nucleic acids of the invention (either nucleic acids or proteins, or both). For example, the integrated systems can include the foregoing software having the appropriate character string information, e.g., used in conjunction with a user interface (e.g., a GUI in a standard operating system such as a Windows, Macintosh or LINUX system) to manipulate strings of characters. As noted, specialized alignment programs such as BLAST can also be incorporated into the systems of the invention for alignment of nucleic acids or proteins (or corresponding character strings).

[0126] Systems for analysis in the present invention typically include a digital computer with an appropriate data base and a sequence of the invention. Software for

aligning sequences, as well as data sets entered into the software system comprising any of the sequences herein can be a feature of the invention. The computer can be, *e.g.*, a PC (Intel x86 or Pentium chip- compatible DOS™, OS2™ WINDOWS™ WINDOWS NT™, WINDOWS95™, WINDOWS98™ , WINDOWS2000, WINDOWSME, or LINUX based machine, a MACINTOSH™, Power PC, or a UNIX based (*e.g.*, SUN™ work station or LINUX based machine) or other commercially common computer which is known to one of skill. Software for entering and aligning or otherwise manipulating sequences is available, or can easily be constructed by one of skill using a standard programming language such as Visualbasic, Fortran, Basic, Java, or the like.

[0127] Any controller or computer optionally includes a monitor which is often a cathode ray tube ("CRT") display, a flat panel display (*e.g.*, active matrix liquid crystal display, liquid crystal display, etc.), or others. Computer circuitry is often placed in a box which includes numerous integrated circuit chips, such as a microprocessor, memory, interface circuits, and others. The box also optionally includes a hard disk drive, a floppy disk drive, a high capacity removable drive such as a writeable CD-ROM, and other common peripheral elements. Inputting devices such as a keyboard or mouse optionally provide for input from a user and for user selection of sequences to be compared or otherwise manipulated in the relevant computer system.

[0128] The computer typically includes appropriate software for receiving user instructions, either in the form of user input into a set parameter fields, *e.g.*, in a GUI, or in the form of preprogrammed instructions, *e.g.*, preprogrammed for a variety of different specific operations. The software then converts these instructions to appropriate language for instructing the operation of the fluid direction and transport controller to carry out the desired operation.

[0129] The software can also include output elements for controlling nucleic acid synthesis (*e.g.*, based upon a sequence or an alignment of a sequences herein) or other operations which occur downstream from an alignment or other operation performed using a character string corresponding to a sequence herein.

Cell Rescue--Treatement

[0130] In one aspect, the invention includes rescue of a cell that is defective in function of one or more endogenous hT1Rx genes or polypeptides. This can be

accomplished simply by introducing a new copy of the gene (or a heterologous nucleic acid that expresses the relevant protein) into a cell. Other approaches, such as homologous recombination to repair the defective gene (e.g., via chimeraplasty) can also be performed. In any event, rescue of function can be measured, e.g., in any of the in vitro assays noted herein. Indeed, this can be used as a general method of screening cells in vitro for an hT1Rx activity. Accordingly, in vitro rescue of function is useful in this context for the myriad in vitro screening methods noted above, e.g., for the identification of sweet or glutamate tastants in cells. The cells that are rescued can include cells in culture, (including primary or secondary cell culture from patients, as well as cultures of well-established cells). Where the cells are isolated from a patient, this has additional diagnostic utility in establishing which hT1Rx sequence is defective in a patient that presents with a tasting defect.

[0131] In another aspect, the cell rescue occurs in a patient, e.g., a human or veterinary patient, e.g., to remedy a tastant defect (for example, older patients often present with an inability to perceive sweet tastants and there are genetic defects that also present as an inability to taste sweet tastants). Thus, one aspect of the invention is gene therapy to remedy tasting defects (or even simply to enhance tastant discrimination), in human or veterinary applications. In these applications, the nucleic acids of the invention are optionally cloned into appropriate gene therapy vectors (and/or are simply delivered as naked or liposome-conjugated nucleic acids), which are then delivered (generally topically to the taste buds, but optionally systemically), optionally in combination with appropriate carriers or delivery agents. Proteins can also be delivered directly, but delivery of the nucleic acid is typically preferred in applications where stable expression is desired.

[0132] Compositions for administration, e.g., comprise a therapeutically effective amount of the gene therapy vector or other relevant nucleic acid, and a pharmaceutically acceptable carrier or excipient. Such a carrier or excipient includes, but is not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and/or combinations thereof. The formulation is made to suit the mode of administration. In general, methods of administering gene therapy vectors for topical use are well known in the art and can be applied to administration of the nucleic acids of the invention.

[0133] Therapeutic compositions comprising one or more nucleic acid of the invention are optionally tested in one or more appropriate in vitro and/or in vivo animal

model of disease, to confirm efficacy, tissue metabolism, and to estimate dosages, according to methods well known in the art. In particular, dosages can initially be determined by activity, stability or other suitable measures of the formulation.

[0134] Administration is by any of the routes normally used for introducing a molecule into ultimate contact with taste bud cells, though topical administration or direct injection into the taste buds is simplest and therefore preferred. The nucleic acids of the invention are administered in any suitable manner, optionally with one or more pharmaceutically acceptable carriers. Suitable methods of administering such nucleic acids in the context of the present invention to a patient are available, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective action or reaction than another route.

[0135] Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present invention. Compositions can be administered by a number of routes including, but not limited to: oral (in this case, topical and oral can be the same or different, e.g., topical delivery to the taste buds can be oral, as can systemic administration by the GI tract), intravenous, intraperitoneal, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal administration. Compositions can be administered via liposomes (e.g., topically), or via topical delivery of naked DNA or viral vectors. Such administration routes and appropriate formulations are generally known to those of skill in the art.

[0136] The compositions, alone or in combination with other suitable components, can also be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can

include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations of packaged nucleic acid can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

[0137] The dose administered to a patient, in the context of the present invention, is sufficient to effect a beneficial therapeutic response in the patient over time, or, e.g., to provide sweet or glutamate tastant discrimination as perceived by the patient in an objective sweet or glutamate tastant test. The dose is determined by the efficacy of the particular vector, or other formulation, and the activity, stability or serum half-life of the polypeptide which is expressed, and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose is also determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular vector, formulation, or the like in a particular patient. In determining the effective amount of the vector or formulation to be administered in the treatment of disease, the physician evaluates local expression in the taste buds, or circulating plasma levels, formulation toxicities, progression of the relevant disease, and/or where relevant, the production of antibodies to proteins encoded by the polynucleotides. The dose administered, e.g., to a 70 kilogram patient are typically in the range equivalent to dosages of currently-used therapeutic proteins, adjusted for the altered activity or serum half-life of the relevant composition. The vectors of this invention can supplement treatment conditions by any known conventional therapy.

[0138] For administration, formulations of the present invention are administered at a rate determined by the LD-50 of the relevant formulation, and/or observation of any side-effects of the vectors of the invention at various concentrations, e.g., as applied to the mass or topical delivery area and overall health of the patient. Administration can be accomplished via single or divided doses.

[0139] If a patient undergoing treatment develops fevers, chills, or muscle aches, he/she receives the appropriate dose of aspirin, ibuprofen, acetaminophen or other pain/fever controlling drug. Patients who experience reactions to the compositions, such as fever, muscle aches, and chills are premedicated 30 minutes prior to the future infusions with either aspirin, acetaminophen, or, e.g., diphenhydramine. Meperidine is used for more severe chills and muscle aches that do not quickly respond to antipyretics and

antihistamines. Treatment is slowed or discontinued depending upon the severity of the reaction.

Kits

[0140] In an additional aspect, the present invention provides kits embodying the methods, composition, systems or apparatus herein. Kits of the invention optionally comprise one or more of the following: (1) a composition, system, system component as described herein; (2) instructions for practicing the methods described herein, and/or for using the compositions or operating the system or system components herein; (3) one or more hT1Rx composition or component; (4) a container for holding components or compositions, and, (5) packaging materials.

EXAMPLES

[0141] The following examples are offered to illustrate, but not to limit the present invention.

[0142] A search of the human genome database led to the identification of three human candidate taste receptors, hT1R1, hT1R2, and hT1R3, which contain seven transmembrane domains. All three genes map to a region of chromosome 1, which is syntenous to the distal end of chromosome 4 in mouse, which contains the *Sac* locus that is involved in detecting sweet tastants. A genetic marker, DVL1, which is linked to the *Sac* locus, is within 1,700 bp of to human T1R3. All three hT1Rs genes are all expressed selectively in human taste receptor cells in the fungiform papillae, consistent with their role in taste perception.

[0143] Accordingly, a family of putative human taste receptors, responsible for detecting sweet tastants are identified. All three hT1Rs sequences are closely related to candidate mammalian sweet taste receptors and sensory receptors (Hoon et al. (1999) *Cell* 96, 541-551; Montmayeur et al. (2001) *Nature Neuroscience*, 4, 492-498; Max et al. (2001) *Nature Genetics*, 28, 58-63; Brown et al. (1993) *Nature* 366, 575-580; and Matsunami et al. (1997) *Cell* 90, 775-784); all three hT1Rs contain seven-transmembrane domains, consistent with previous studies implicating G proteins in sweet taste transduction. Furthermore, all three hT1Rs localize in human chromosome 1, in accord with recent studies that show most functionally related chemosensory receptors tend to cluster in the same region of the chromosome. The mouse syntenic locus of hT1R3 is very close to the *Sac* locus, which has

been implicated in sweet taste transduction (Fuller, J. (1974) *J.Hered.* 65, 33-36; Lush et al. (1995) *Genet. Res.* 66,167-174; Bachmanov, A., (1997) *Mamm. Genome* 8, 545-548). Finally, the hT1Rs are specifically expressed in subsets of taste receptor cells in human tongue.

[0144] The results described here suggest that the T1R3 gene is responsible for the *Sac* phenotype (*See also*, Montmayer (2001) and Max (2001), *above* and Nelson, et al. (2001) *Cell* 106, 381-390).

[0145] Interestingly, in contrast to observations that shows rat T1R1 and T1R2 are expressed in same taste buds, but in most cases, not in the same cells (Hoon (1999), *above*), it was found that hT1R2 and hT1R3 are expressed in most of cases in the same taste cells. Thus, a single taste cell may express more than one type of taste receptors (Adler (2000), *above*), consistent with the experimental observations suggesting that some taste cells may respond to, but not discriminate, multiple taste stimuli (Lindemann, B. (1996) *Physiol.Rev.* 76, 718-766). This is in striking contrast to olfactory and vomeronasal systems, in which each receptor cell only expresses one receptor gene, providing a cellular mechanism for stimuli discrimination (Buck, L. (2000) *Cell* 100, 611-6). The co-expression of large subsets of T2Rs and T1Rs in individual taste cells, together with the observation that each sensory fiber innervates multiple taste buds and several taste cells within each taste bud, would result in detection of a large range of distinct tastants, but would not allow discrimination of these substances. The fact that hT1R2 and hT1R3 only share 25% sequence identity suggests distinct ligand specificity. The co-expression of hT1R2 and hT1R3 in the same taste cell reflect the possibility of heterodimer formation, which can lead to different ligand specificity relative to that of each receptor, as is the cases for many GPCR dimers. The results herein show that hT1R1 is expressed in different cells relative to hT1R2 and hT1R3.

[0146] For additional evidence that the mouse T1R2 and T1R3 combine to function as sweet receptor, and mouse T1R3 rescued the *Sac* phenotype, *see* (Nelson et al. (2001) *Cell* 106, 381-390). Further confirming the results herein, it has also recently been shown that the human T1R2/T1R3 recognizes diverse natural and synthetic sweeteners and that human T1R1/T1R3 responds to the umami taste stimulus l-glutamate (Li et al. (2002) *Proc. Natl. Acad. Sci.* 99, 4692-4696).

MATERIALS AND METHODS

hT1R Gene Searching

[0147] The Framesearch program (protein query searches translated protein database) was used to search the Celera human genome database (Release R18 to R25) using rat T1R1 protein as the query. After filtering sequences containing either stop codon(s) or known genes, the contigs containing potential novel genes were submitted to Genescan (http://gnes.mit.edu/GENESCAN.html) for full-length gene prediction. For those exons that were missed by Genescan, TBlastN searches were applied to the same contig using rat T1R1 as the query. All novel protein sequences were subjected to a membrane domain prediction program (TopPhred 2) for verification.

[0148] The 5' end of human T1R2 was obtained by cDNA PCR. The oligonucleotide, 5'-CGCAGCAAAGCCGGGAAGCGCACCTTGTCTC-3' (SEQ ID NO: 7) corresponding to nucleotides 515-545 of hT1R2, was used for cDNA PCR using Marathon-Ready cDNA as template (Clontech). A 600 bp fragment was obtained and cloned into Topo-2.1 vector for sequencing (Invitrogen). The deduced amino acid sequence was then assembled with the Genescan-predicted hT1R2 sequence.

Chromosome Mapping

[0149] The coding regions of hT1R1 and hT1R3 were used as queries to search the NCBI human genome database (http://www.ncbi.nlm.nih.gov/genome /seq/page.cgi?F= HsBlast.html &&ORG=Hs) to obtain the chromosome locations relative to telomere. Because the sequence of hT1R2 was not in the NCBI database, a fragment sequence from Celera contig x2HTBKLHUGU that contains the hT1R2 gene was used to search the HTGS database. A BAC clone, AL080251, was found and a search of the human genome database identified its chromosome location. Because one end of the BAC clone AL080251 was about 30 kb away from the hT1R2 gene (the putative third exon), the chromosome location of hT1R2 was deduced from its location relative to the BAC clone AL080251. The genetic marker DVL1 was initially obtained from the NCBI human genome database and used to identify the corresponding location in the mouse syntenic region from the Jackson laboratory Mouse Informatics Database (http://www.informatics.jax.org/ menus/homology_menu.shtml). The chromosome locations of mT1Rs were also obtained from the Jackson laboratory Mouse Informatics Database.

In Situ Hybridization

[0150] Human tongue tissue was obtained from a donor of 70 year old male Caucasian (National Disease Research Interchange). Fresh frozen sections (10 micrometer) of taste papillae were hybridized to digoxigenin-labelled cRNA probes prepared from cloned segments of cDNA encoding the last exons of hT1R1-3. All hybridizations were carried out at high stringency (5XSSC, 50% formamide, 55°C). For single-label detection, signals were developed using alkaline phosphatase-conjugated antibodies to digoxigenin and NBT/BCIP substrate (Roshe). For two-color fluorescent *in situ* hybridization, sections of taste papillae were hybridized simultaneously to both digoxigenin- (hT1R2) and fluorescein-(hT1R3) labeled cRNA probes (Roche). Following hybridization, the labeled probes were recognized with peroxidase-anti-digoxigenin and alkaline phosphatase-anti-fluorescein antibodies, respectively (Roche). The tyramide-biotin/streptavidin-Alexa 488 (NEN and Molecular Probe) and HNPP/fast red (Roche) were then used as substrates for fluorescent labeling with peroxidase and alkaline phosphatase, respectively. Sections were mounted in VECTASHIELD Mounting Medium with DAPI (VECTOR Laboratories) to counterstain nuclei.

[0151] In one experiment, expression of the three sweet receptor mRNAs in human taste cells was analyzed. Frozen sections of human fungiform taste papillae were hybridized with digoxigenin-labelled hT1R1, hT1R2, hT1R3 cRNA probes in either anti-sense or sense orientation. The level of expression of hT1R1 was observed to be very low compared to that of hT1R2 and T1R3. The papillae from an adjacent section hybridized to the sense probe and showed no non-specific binding.

[0152] In another experiment, it was determined that hT1R2 colocalizes with hT1R3 in human taste receptor cells. Papillae from human fungiform were hybridized simultaneously with a digoxigenin-labelled hT1R2 and a fluorescein-labelled T1R3 probe. The digoxigenin-labelled T1R2 probe and fluorescein-labeled T1R3 probe were imaged with Alexa 488 (green) and HNPP/fast red (red), respectively. The overlay of the two images shows that some cells coexpress T1R2 and T1R3 (yellow).

EXAMPLE 1
Identification of Human Sweet Receptor Genes

[0153] A series of search/verification criteria were initially developed as part of the search procedure. The search was carried out using both DNA and protein sequences as queries to increase the possibility of discovering new genes in the human genome. The candidate fragments/genes were evaluated based on existing knowledge of GPCRs and taste receptors, i.e., the sequences of sweet receptors are related to each other; the deduced amino acid sequences should show seven transmembrane domains; and the sweet receptors should be clustered in the same chromosome region. Rat T1R1 (rT1R1) was first utilized as the query to search all public genome and EST databases. No homologous sequences were found initially. The Celera human genome database in an unassembled version was then searched, using the Framesearch program. More than twenty fragments encoding peptides showing similarity to rT1R1 protein were discovered. PCR was used to assess the expression of these fragments. Seven fragments were expressed in testis. Although it is possible that these fragments come from the same gene, the fact that the several different peptides encoded by these fragments show homology to the same region of rT1R1 suggest that there may be several T1R1 homologues in human.

[0154] After the small DNA fragments were assembled into larger fragments, the database was searched again. Based on similarity scores, eleven sequences were chosen for further evaluation. Of these eleven sequences, five fragments were excluded because they contain stop codons in the coding regions, suggesting that they might be pseudogenes. The remaining six fragments were further characterized. Of these, two fragments correspond to two known genes-metabotropic glutamate receptor 3 and Ca^{2+} sensor 5, and three encode peptides that are homologous to rat T1R1 and are localized in chromosome 1 (see below). The full-length coding region of these three genes was predicted from their corresponding contigs (x8YLHLD for putative hT1R1, x2HTBKLHUGU for putative hT1R2, x2HTBKWRET8 for putative hT1R3) by using the Genscan gene prediction program and tBlastN with rat T1R1 as the query. Two full-length genes encoding proteins with seven transmembrane helices are predicted. The third gene, which encodes a peptide more closely related to rat T1R2, lacks approximately 150 amino acids at the N-terminus due to the fact that the contig x2HTBKLHUGU has several un-sequenced gaps in the putative exon 1 and 2

coding-regions. The EST database was also searched to find any ESTs corresponding to hT1Rs, but none were found, suggesting tissue-specific and/or low-level expressions.

[0155] The PCR method was then utilized to obtain the 5' sequence of the putative human T1R2 cDNA. Using a gene-specific primer, a 600 bp fragment was obtained from human testis cDNA template. Sequencing revealed an in-frame-peptide that is very similar to the N-terminal 150 amino acids of rat T1R2 N-terminus, strongly suggesting this to be 5' sequences of hT1R2 cDNA.

[0156] The deduced amino acid sequences of all three human T1Rs show a high degree of homology to both their mouse and rat counterparts (Figure 1). hT1R1 shows much higher sequence identity to its orthologoue, mT1R1 in mouse and rT1R1 in rat (69.8% and 70.0% amino acid identity, respectively) than its homologues, hT1R2 and hT1R3 (30.7% and 26.0%, respectively). The same is true for the other two members: hT1R2 shows 67.9% and 70.4% amino acid identity to mT1R2 and rT1R2, respectively; hT1R3 shows 72% identity to mT1R3. This group of human taste receptors belongs to GPCR subfamily 3, which includes metabotropic glutamate receptors, extracellular Ca^{2+} sensors, and pheromone receptors. All three hT1Rs have long N-terminal extracellular domains (Figure 1), similar to other members of this family of GPCRs. This long N-terminal extracellular domain has been suggested to function in dimerization and/or ligand binding as described, e.g., in Kunishima et al., *Nature* 407: 971-977, 2000. The nucleotide sequences of the hT1R1, hT1R2 and hT1R3 cDNAs are shown in Figures 2A, 2B, and 2C, respectively.

[0157] The three hT1Rs are encoded by a similar number of exons, hT1R1 and hT1R3 by 6 exons, and hT1R2 by more than 5 exons. This result is consistent with that of the mouse T1Rs, as described by Montmayeur et al., *Nature Neuroscience* 4:492-498, 2001. However, the hT1Rs genes span different sizes in the chromosome: the hT1R1 coding region spans 24 kb; hT1R2 occupies more than 15kb, and hT1R3 is only 4kb in size (Figure 3, see below). Interestingly, all the transmembrane domains are encoded by the last and also the largest exon for all three hT1Rs.

EXAMPLE 2

Mapping of the Human T1Rs Receptor Genes To a Region in Chromosome 1, the Syntenic Region of Mouse Distal Chromosome 4 End Containing the *Sac* Locus

[0158] We then asked whether the human T1Rs co-localize to the same chromosome, as might be expected for taste receptors having similar properties. Using hT1R1 to search the human genome database in NCBI, the hT1R1 gene was found to be localized in the contig NT_019267, which maps to chromosome 1. The coding region of hT1R1 spans 24 kb from 12433K to 12409K of chromosome 1 (Figure 3). Unfortunately, hT1R2 was not able to be mapped directly because there is no corresponding clone in the NCBI human genome database. An electronic chromosome walking strategy was used to find overlapping clones. Using a sequence in the region of 2.16 Mp from Celera contig x2HTBKLHUGU, an overlapping BAC clone, AL080251 was found, which has been assigned to chromosome 1p35.2-p36.23. The end of the AL080251 clone, 30kb from the hT1R2 gene, maps to a position of 13804K in chromosome 1. The location of hT1R was at 13776K to 13761K in chromosome 1 (Figure 3). Using the same approach for hT1R1, hT1R3 was found to be localized to a region of 4 kb, from 61116K to 61111K in human chromosome 1 (Figure 3). This region belongs to contig NT_025635. To find the locus information, the human high-throughput genome project database (htgs) was also searched and hT1R3 was found in two BAC clones, AC026283 and AL139287. These two BAC clones, however, have not been assigned to a locus in the chromosome. We then used the electronic chromosome walking strategy again to find a overlapping BAC clone, AL391244.11, which overlaps with AC026283 and is assigned to human chromosome 1p36.31-36.33.

[0159] The above results show that all three human T1Rs indeed form a cluster in chromosome 1. Using The Jackson Laboratory Mouse Informatics database, the corresponding region in mouse was determined to be distal chromosome 4. Interestingly, the *Sac* locus has been mapped to the same distal region of chromosome 4 at about 83 cM, as described by Fuller, *J. Hered.* 65:33-36, 1974; Lush et al. Genet. Res. 66:167-174, 1995; and Bachmanov, *Mamm.Genome* 8:545-548, 1997. Recently, mT1R1 has also been mapped to this region, approximately 5cM from the *Sac* locus as described, e.g., in Li et al., *Mamm. Genome* 12: 13-15, 2001. hT1R1 shows very high sequence similarity to mT1R1 (69.8%,

see above). These results suggest that there might be a sweet receptor cluster in this region. To determine whether any of the hT1Rs identified may be an orthologue of *Sac* locus, several genetic markers closely linked to hT1Rs were examined. One of the markers, DVL1-a human dishevelled homologue, which is tightly linked to the hT1R3 gene only about 1,700 bp away, was found to map to the distal end of chromosome 4 at 82.0 cM. This location is very close to the mapped *Sac* locus at 83cM, suggesting the likelihood of T1R3 as a gene of the *Sac* locus. Recently, two papers have been published which also suggest that T1R3 is the closest GPCR gene to *Sac* locus (see, e.g., Montmayeur et al., *supra*; and Max et al., *Nature Genetics* 28:58-63, 2001.

EXAMPLE 3
Expression of hT1Rs in Taste Cells

[0160] If hT1Rs are taste receptors, they should be expressed in taste tissues. According to classical models of taste discrimination, fungiform papillae are more sensitive to sweet substances than other regions of the tongue. To examine the expression of the hT1Rs, *in situ* hybridizations were carried out with sections containing human fungiform taste papillae. All three hT1Rs genes were found to be selectively expressed in a subset of taste receptor cells, but absent from surrounding lingual epithelium. Control sense cRNA probes did not hybridize to the taste cells in the immediate adjacent sections. The hT1R2 and hT1R3 probes hybridize to approximately 10-20% of taste cells. The hybridization signal for hT1R1 was much weaker than those for hT1R2 and hT1R3 in fungiform papillae. The hybridization signals for hT1R1 were also very weak in circumvallate and foliate taste papillae. These results are consistent with those described for the recently published mouse T1Rs as described in Montmayeur et al., *supra*.

[0161] A preliminary analysis of the expression pattern of the hT1Rs was also carried out. In most cases, T1R1 was expressed in different taste buds from that of hT1R2 and hT1R3, consistent with the previous studies for rat rT1R1 and rT1R2 (see Hoon et al., *supra*). Surprisingly, hT1R2 and hT1R3 are expressed in the same taste bud in single-labeling *in situ* experiments. To examine whether hT1R2 and hT1R3 might be expressed in the same taste cells, a fungiform papillae section was hybridized with different labeled-hT1R2 and hT1R3 cRNA probes simultaneously. The results from the hybridization studies show that hT1R2 and hT1R3 are expressed largely in the same taste cells (5 of 5 taste buds

examined in the section). However, some T1R2-expressing cells do not express T1R3. These results are in contrast to a recent observation that mouse all T1R2-expressing cells also express T1R3 (*see also*, Montmayeur et al., *supra*).

[0162] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. Thus, the above description should not be construed as limiting, but merely as exemplification of preferred embodiments.

[0163] All patent applications, patents and literature references cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if every such patent application, patent, or literature reference were indicated to be incorporated by reference in its entirety.

SEQUENCE TABLE

Example T1Rx Nucleic Acids And Polypeptides

>hT1R1 (Nucleic Acid; SEQ ID NO:10)

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>hT1R1 (amino acid; SEQ ID NO:1)

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>hT1R2 (nucleic acid; SEQ ID NO: 11)

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>hT1R2 (amino acid; SEQ ID NO: 4)
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CCGCTCACGGGCTGCCTGAGCACACTCTTCCTGCAGGCGGCCGAGATCTTCGTGGAGTCAGAACTGCCTCTGAGCTGGGC
AGACCGGCTGAGTGGCTGCCTGCGGGGGCCCTGGGCCTGGCTGGTGGTGCTGCTGGCCATGCTGGTGGAGGTCGCACTGT
GCACCTGGTACCTGGTGGCCTTCCCGCCGGAGGTGGTGACGGACTGGCACATGCTGCCCACGGAGGCGCTGGTGCACTGC
CGCACACGCTCCTGGGTCAGCTTCGGCCTAGCGCACGCCACCAATGCCACGCTGGCCTTTCTCTGCTTCCTGGGCACTTT
CCTGGTGCGGAGCCAGCCGGGCCGCTACAACCGTGCCCGTGGCCTCACCTTTGCCATGCTGGCCTACTTCATCACCTGGG
TCTCCTTTGTGCCCCTCCTGGCCAATGTGCAGGTGGTCCTCAGGCCCGCCGTGCAGATGGGCGCCCTCCTGCTCTGTGTC
CTGGGCATCCTGGCTGCCTTCCACCTGCCCAGGTGTTACCTGCTCATGCGGCAGCCAGGGCTCAACACCCCCGAGTTCTT
CCTGGGAGGGGGCCCTGGGGATGCCCAAGGCCAGAATGACGGGAACACAGGAAATCAGGGGAAACATGAGTGA

>hT1R3 (amino acid; SEQ ID NO: 7)
MLGPAVLGLSLWALLHPGTGAPLCLSQQLRMKGDYVLGGLFPLGEAEEAGLRSRTRPSSPVCTRFSSNGLLWALAMKMAV
EEINNKSDLLPGLRLGYDLFDTCSEPVVAMKPSLMFLAKAGSRDIAAYCNYTQYQPRVLAVIGPHSSELAMVTGKFFSFF
LMPQVSYGASMELLSARETFPSFFRTVPSDRVQLTAAAELLQEFGWNWVAALGSDDEYGRQGLSIFSALAAARGICIAHE
GLVPLPRADDSRLGKVQDVLHQVNQSSVQVVLLFASVHAAHALFNYSISSRLSPKVWVASEAWLTSDLVMGLPGMAQMGT
VLGFLQRGAQLHEFPQYVKTHLALATDPAFCSALGEREQGLEEDVVGQRCPQCDCITLQNVSAGLNHHQTFSVYAAVYSV
AQALHNTLQCNASGCPAQDPVKPWQLLENMYNLTFHVGGLPLRFDSSGNVDMEYDLKLWVWQGSVPRLHDVGRFNGSLRT
ERLKIRWHTSDKPVSRCSRQCQEGQVRRVKGFHSCCYDCVDCEAGSYRQNPDDIACTFCGQDEWSPERSTRCFRRRSRFL
AWGEPAVLLLLLLLSLALGLVLAAILGLFVHHRDSPLVQASGGPLACFGLVCLGLVCLSVLLFPGQPSPARCLAQQPLSHL
PLTGCLSTLFLQAAEIFVESELPLSWADRLSGCLRGPWAWLVVLLAMLVEVALCTWYLVAFPPEVVTDWHMLPTEALVHC
RTRSWVSFGLAHATNATLAFLCFLGTFLVRSQPGRYNRARGLTFAMLAYFITWVSFVPLLANVQVVLRPAVQMGALLLCV
LGILAAFHLPRCYLLMRQPGLNTPEFFLGGGPGDAQGQNDGNTGNQGKHE.

>mT1R1 (Nucleic Acid; SEQ ID NO. 13)
ATGCTTTTCTGGGCAGCTCACCTGCTGCTCAGCCTGCAGCTGGCCGTTGCTTACTGCTGGGCTTTCAGCTGCCAAAGGAC
AGAATCCTCTCCAGGTTTCAGCCTCCCTGGGGACTTCCTCCTGGCAGGCCTGTTCTCCCTCCATGCTGACTGTCTGCAGG
TGAGACACAGACCTCTGGTGACAAGTTGTGACAGGTCTGACAGCTTCAACGGCCATGGCTATCACCTCTTCCAAGCCATG
CGGTTCACCGTTGAGGAGATAAACAACTCCACAGCTCTGCTTCCCAACATCACCCTGGGGTATGAACTGTATGACGTGTG
CTCAGAGTCTTCCAATGTCTATGCCACCCTGAGGGTGCCCGCCCAGCAAGGGACAGGCCACCTAGAGATGCAGAGAGATC
TTCGCAACCACTCCTCCAAGGTGGTGGCACTCATTGGGCCTGATAACACTGACCACGCTGTCACCACTGCTGCCCTGCTG
AGCCCTTTTCTGATGCCCCTGGTCAGCTATGAGGCGAGCAGCGTGATCCTCAGTGGGAAGCGCAAGTTCCCGTCCTTCTT
GCGCACCATCCCCAGCGATAAGTACCAGGTGGAAGTCATAGTGCGGCTGCTGCAGAGCTTCGGCTGGGTCTGGATCTCGC
TCGTTGGCAGCTATGGTGACTACGGGCAGCTGGGCGTACAGGCGCTGGAGGAGCTGGCCACTCCACGGGGCATCTGCGTC
GCCTTCAAGGACGTGGTGCCTCTCTCCGCCCAGGCGGGTGACCCAAGGATGCAGCGCATGATGCTGCGTCTGGCTCGAGC
CAGGACCACCGTGGTCGTGGTCTTCTCTAACCGGCACCTGGCTGGAGTGTTCTTCAGGTCTGTGGTGCTGGCCAACCTGA
CTGGCAAAGTGTGGATCGCCTCCGAAGACTGGGCCATCTCCACGTACATCACCAATGTGCCCGGGATCCAGGGCATTGGG
ACGGTGCTGGGGGTGGCCATCCAGCAGAGACAAGTCCCTGGCCTGAAGGAGTTTGAAGAGTCCTATGTCCAGGCAGTGAT
GGGTGCTCCCAGAACTTGCCCAGAGGGGTCCTGGTGCGGCACTAACCAGCTGTGCAGGGAGTGTCACGCTTTCACGACAT
GGAACATGCCCGAGCTTGGAGCCTTCTCCATGAGCGCTGCCTACAATGTGTATGAGGCTGTGTATGCTGTGGCCCACGGC
CTCCACCAGCTCCTGGGATGTACCTCTGGGACCTGTGCCAGAGGCCCAGTCTACCCCTGGCAGCTTCTTCAGCAGATCTA
CAAGGTGAATTTCCTTCTACATAAGAAGACTGTAGCATTCGATGACAAGGGGGACCCTCTAGGTTATTATGACATCATCG

CCTGGGACTGGAATGGACCTGAATGGACCTTTGAGGTCATTGGTTCTGCCTCACTGTCTCCAGTTCATCTAGACATAAAT
AAGACAAAAATCCAGTGGCACGGGAAGAACAATCAGGTGCCTGTGTCAGTGTGTACCAGGGACTGTCTCGAAGGGCACCA
CAGGTTGGTCATGGGTTCCCACCACTGCTGCTTCGAGTGCATGCCCTGTGAAGCTGGGACATTTCTCAACACGAGTGAGC
TTCACACCTGCCAGCCTTGTGGAACAGAAGAATGGGCCCCTGAGGGGAGCTCAGCCTGCTTCTCACGCACCGTGGAGTTC
TTGGGGTGGCATGAACCCATCTCTTTGGTGCTATTAGCAGCTAACACGCTATTGCTGCTGCTGCTGATTGGGACTGCTGG
CCTGTTTGCCTGGCGTCTTCACACGCCTGTTGTGAGGTCAGCTGGGGGTAGGCTGTGCTTCCTCATGCTGGGTTCCTTGG
TAGCTGGGAGTTGCAGCCTCTACAGCTTCTTCGGGAAGCCCACGGTGCCCGCGTGCTTGCTGCGTCAGCCCCTCTTTTCT
CTCGGGTTTGCCATTTTCCTCTCCTGTCTGACAATCCGCTCCTTCCAACTGGTCATCATCTTCAAGTTTTCTACCAAGGT
ACCCACATTCTACCACACTTGGGCCCAAAACCATGGTGCCGGAATATTCGTCATTGTCAGCTCCACGGTCCATTTGTTCC
TCTGTCTCACGTGGCTTGCAATGTGGACCCCACGGCCCACCAGGGAGTACCAGCGCTTCCCCCATCTGGTGATTCTTGAG
TGCACAGAGGTCAACTCTGTGGGCTTCCTGGTGGCTTTCGCACACAACATCCTCCTCTCCATCAGCACCTTTGTCTGCAG
CTACCTGGGTAAGGAACTGCCGGAGAACTATAACGAAGCCAAATGTGTCACCTTCAGCCTGCTCCTCCACTTCGTATCCT
GGATCGCTTTCTTCACCATGTCCAGCATTTACCAGGGCAGCTACCTACCCGCGGTCAATGTGCTGGCAGGGCTGGCCACT
CTGAGTGGCGGCTTCAGCGGCTATTTCCTCCCTAAATGCTACGTGATTCTCTGCCGTCCAGAACTCAACAACACAGAACA
CTTTCAGGCCTCCATCCAGGACTACACGAGGCGCTGCGGCACTACCTGA

>mT1R1 (Amino Acid; SEQ ID NO: 2)
MLFWAAHLLLSLQLAVAYCWAFSCQRTESSPGFSLPGDFLLAGLFSLHADCLQVRHRPLVTSCDRSDSFNGHGYHLFQAM
RFTVEEINNSTALLPNITLGYELYDVCSESSNVYATLRVPAQQGTGHLEMQRDLRNHSSKVVALIGPDNTDHAVTTAALL
SPFLMPLVSYEASSVILSGKRKFPSFLRTIPSDKYQVEVIVRLLQSFGWVWISLVGSYGDYGQLGVQALEELATPRGICV
AFKDVVPLSAQAGDPRMQRMMLRLARARTTVVVVFSNRHLAGVFFRSVVLANLTGKVWIASEDWAISTYITNVPGIQGIG
TVLGVAIQQRQVPGLKEFEESYVQAVMGAPRTCPEGSWCGTNQLCRECHAFTTWNMPELGAFSMSAAYNVYEAVYAVAHG
LHQLLGCTSGTCARGPVYPWQLLQQIYKVNFLLHKKTVAFDDKGDPLGYYDIIAWDWNGPEWTFEVIGSASLSPVHLDIN
KTKIQWHGKNNQVPVSVCTRDCLEGHHRLVMGSHHCCFECMPCEAGTFLNTSELHTCQPCGTEEWAPEGSSACFSRTVEF
LGWHEPISLVLLAANTLLLLLLIGTAGLFAWRLHTPVVRSAGGRLCFLMLGSLVAGSCSLYSFFGKPTVPACLLRQPLFS
LGFAIFLSCLTIRSFQLVIIFKFSTKVPTFYHTWAQNHGAGIFVIVSSTVHLFLCLTWLAMWTPRPTREYQRFPHLVILE
CTEVNSVGFLVAFAHNILLSISTFVCSYLGKELPENYNEAKCVTFSLLLHFVSWIAFFTMSSIYQGSYLPAVNVLAGLAT
LSGGFSGYFLPKCYVILCRPELNNTEHFQASIQDYTRRCGTT.

>mT1R2 (Nucleic Acid; SEQ ID NO: 14)
ATGCTGCGCACTGTGCCCAGCGCCACCCACCACATCGAGGCCATGGTGCAACTGATGGTTCACTTCCAGTGGAACTGGAT
CGTGGTGCTGGTGAGCGATGACGATTATGGCCGAGAGAACAGCCACCTGCTGAGCCGAGCGACCTGACCAACACTGGCGATA
TCTGCATTGCCTTCCAGGAGGTTCTGCCTGTACCAGAACCCAACCAGGCCGTGAGGCCTGAGGAGCAGGACCAACTGGAC
AACATCCTGGACAAGCTGCGGCGGACCTCGGCGCGTGTGGTGGTGATATTCTCGCCAGAGCTGAGCCTGCACAACTTCTT
CCGCGAGGTGCTGCGCTGGAACTTCACAGGCTTTGTGTGGATTGCCTCTGAGTCCTGGGCCATCGACCCTGTTCTACACA
ACCTCACAGAGCTGCGCCACACGGGCACTTTCCTGGGCGTCACCATCCAGAGGGTGTCCATCCCTGGCTTCAGCCAGTTC
CGAGTGCGCCACGACAAGCCAGAGTATCCCATGCCTAACGAGACCAGCCTGAGGACTACCTGTAACCAGGACTGTGACGC
CTGCATGAACATCACCGAGTCCTTTAACAACGTTCTCATGCTTTCGGGGGAGCGTGTGGTCTACAGTGTGTACTCGGCCG
TCTACGCGGTAGCCCACACCCTCCACAGACTCCTCCACTGCAACCAGGTCCGCTGCACCAAGCAAATCGTCTATCCATGG
CAGCTACTCAGGGAGATCTGGCATGTCAACTTCACGCTCCTGGGCAACCAGCTCTTCTTCGACGAACAAGGGGACATGCC
GATGCTCCTGGACATCATCCAGTGGCAATGGGGCCTGAGCCAGAACCCCTTCCAAAGCATCGCCTCCTACTCCCCCACCG
AGACGAGGCTGACCTACATTAGCAATGTGTCCTGGTACACCCCCAACAACACGGTCCCCATATCCCATGTGTTCTAAGAGT
TGCCAGCCTGGGCAAATGAAAAAACCCATAGGCCTCCACCCGTGCTGCTTCGAGTGTGTGGACTGTCCGCCGGGCACCTA
CCTCAACCGATCAGTAGATGAGTTTAACTGTCTGTCCTGCCCGGGTTCCATGTGGTCTTACAAGAACAACATCGCTTGCT
TCAAGCGGCGGCTGGCCTTCCTGGAGTGGCACGAAGTGCCCACTATCGTGGTGACCATCCTGGCCGCCCTGGGCTTCATC
AGTACGCTGGCCATTCTGCTCATCTTCTGGAGACATTTCCAGACGCCCATGGTGCGCTCGGCGGGCGGCCCCATGTGCTT
CCTGATGCTGGTGCCCCTGCTGCTGGCGTTCGGGATGGTCCCCGTGTATGTGGGCCCCCCCACGGTCTTCTCCTGTTTCT
GCCGCCAGGCTTTCTTCACCGTTTGCTTCTCCGTCTGCCTCTCCTGCATCACGGTGCGCTCCTTCCAGATTGTGTGCGTC
TTCAAGATGGCCAGACGCCTGCCAAGCGCCTACGGTTTCTGGATGCGTTACCACGGGCCCTACGTCTTTGTGGCCTTCAT
CACGGCCGTCAAGGTGGCCCTGGTGGCAGGCAACATGCTGGCCACCACCATCAACCCCATTGGCCGGACCGACCCCGATG
ACCCCAATATCATAATCCTCTCCTGCCACCCTAACTACCGCAACGGGCTACTCTTCAACACCAGCATGGACTTGCTGCTG
TCCGTGCTGGGTTTCAGCTTCGCGTACGTGGGCAAGGAACTGCCCACCAACTACAACGAAGCCAAGTTCATCACCCTCAG
CATGACCTTCTCCTTCACCTCCTCCATCTCCCTCTGCACGTTCATGTCTGTCCACGATGGCGTGCTGGTCACCATCATGG
ATCTCCTGGTCACTGTGCTCAACTTTCTGGCCATCGGCTTGGGGTACTTTGGCCCCAAGTGTTACATGATCCTTTTCTAC
CCGGAGCGCAACACTTCAGCTTATTTCAATAGCATGATTCAGGGCTACACGATGAGGAAGAGCTAG

>mT1R2 (amino acid; SEQ ID NO: 5)
MLRTVPSATHHIEAMVQLMVHFQWNWIVVLVSDDDYGRENSHLLSQRLTNTGDICIAFQEVLPVPEPNQAVRPEEQDQLD
NILDKLRRTSARVVVIFSPELSLHNFFREVLRWNFTGFVWIASESWAIDPVLHNLTELRHTGTFLGVTIQRVSIPGFSQF
RVRHDKPEYPMPNETSLRTTCNQDCDACMNITESFNNVLMLSGERVVYSVYSAVYAVAHTLHRLLHCNQVRCTKQIVYPW
QLLREIWHVNFTLLGNQLFFDEQGDMPMLLDIIQWQWGLSQNPFQSIASYSPTETRLTYISNVSWYTPNNTVPISMCSKS
CQPGQMKKPIGLHPCCFECVDCPPGTYLNRSVDEFNCLSCPGSMWSYKNNIACFKRRLAFLEWHEVPTIVVTILAALGFI
STLAILLIFWRHFQTPMVRSAGGPMCFLMLVPLLLAFGMVPVYVGPPTVFSCFCRQAFFTVCFSVCLSCITVRSFQIVCV
FKMARRLPSAYGFWMRYHGPYVFVAFITAVKVALVAGNMLATTINPIGRTDPDDPNIIILSCHPNYRNGLLFNTSMDLLL
SVLGFSFAYVGKELPTNYNEAKFITLSMTFSFTSSISLCTFMSVHDGVLVTIMDLLVTVLNFLAIGLGYFGPKCYMILFY
PERNTSAYFNSMIQGYTMRKS.

>mT1R3 (nucleic acid; SEQ ID NO: 15)
ATGCCAGCTTTGGCTATCATGGGTCTCAGCCTGGCTGCTTTCCTGGAGCTTGGGATGGGGGCCTCTTTGTGTCTGTCACA
GCAATTCAAGGCACAAGGGGACTACATACTGGGCGGGCTATTTCCCCTGGGCTCAACCGAGGAGGCCACTCTCAACCAGA
GAACACAACCCAACAGCATCCCGTGCAACAGGTTCTCACCCCTTGGTTTGTTCCTGGCCATGGCTATGAAGATGGCTGTG
GAGGAGATCAACAATGGATCTGCCTTGCTCCCTGGGCTGCGGCTGGGCTATGACCTATTTGACACATGCTCCGAGCCAGT
GGTCACCATGAAATCCAGTCTCATGTTCCTGGCCAAGGTGGGCAGTCAAAGCATTGCTGCCTACTGCAACTACACACAGT
ACCAACCCCGTGTGCTGGCTGTCATCGGCCCCCACTCATCAGAGCTTGCCCTCATTACAGGCAAGTTCTTCAGCTTCTTC
CTCATGCCACAGGTCAGCTATAGTGCCAGCATGGATCGGCTAAGTGACCGGGAAACGTTTCCATCCTTCTTCCGCACAGT
GCCCAGTGACCGGGTGCAGCTGCAGGCAGTTGTGACTCTGTTGCAGAACTTCAGCTGGAACTGGGTGGCCGCCTTAGGGA
GTGATGATGACTATGGCCGGGAAGGTCTGAGCATCTTTTCTAGTCTGGCCAATGCACGAGGTATCTGCATCGCACATGAG
GGCCTGGTGCCACAACATGACACTAGTGGCCAACAGTTGGGCAAGGTGCTGGATGTACTACGCCAAGTGAACCAAAGTAA
AGTACAAGTGGTGGTGCTGTTTGCCTCTGCCCGTGCTGTCTACTCCCTTTTTAGTTACAGCATCCATCATGGCCTCTCAC
CCAAGGTATGGGTGGCCAGTGAGTCTTGGCTGACATCTGACCTGGTCATGACACTTCCCAATATTGCCCGTGTGGGCACT
GTGCTTGGGTTTTTGCAGCGGGGTGCCCTACTGCCTGAATTTTCCCATTATGTGGAGACTCACCTTGCCCTGGCCGCTGA
CCCAGCATTCTGTGCCTCACTGAATGCGGAGTTGGATCTGGAGGAACATGTGATGGGGCAACGCTGTCCACGGTGTGACG

ACATCATGCTGCAGAACCTATCATCTGGGCTGTTGCAGAACCTATCAGCTGGGCAATTGCACCACCAAATATTTGCAACC
TATGCAGCTGTGTACAGTGTGGCTCAAGCCCTTCACAACACCCTACAGTGCAATGTCTCACATTGCCACGTATCAGAACA
TGTTCTACCCTGGCAGCTCCTGGAGAACATGTACAATATGAGTTTCCATGCTCGAGACTTGACACTACAGTTTGATGCTG
AAGGGAATGTAGACATGGAATATGACCTGAAGATGTGGGTGTGGCAGAGCCCTACACCTGTATTACATACTGTGGGCACC
TTCAACGGCACCCTTCAGCTGCAGCAGTCTAAAATGTACTGGCCAGGCAACCAGGTGCCAGTCTCCCAGTGTTCCCGCCA
GTGCAAAGATGGCCAGGTTCGCCGAGTAAAGGGCTTTCATTCCTGCTGCTATGACTGCGTGGACTGCAAGGCGGGCAGCT
ACCGGAAGCATCCAGATGACTTCACCTGTACTCCATGTAACCAGGACCAGTGGTCCCCAGAGAAAAGCACAGCCTGCTTA
CCTCGCAGGCCCAAGTTTCTGGCTTGGGGGGAGCCAGTTGTGCTGTCACTCCTCCTGCTGCTTTGCCTGGTGCTGGGTCT
AGCACTGGCTGCTCTGGGGCTCTCTGTCCACCACTGGGACAGCCCTCTTGTCCAGGCCTCAGGTGGCTCACAGTTCTGCT
TTGGCCTGATCTGCCTAGGCCTCTTCTGCCTCAGTGTCCTTCTGTTCCCAGGGCGGCCAAGCTCTGCCAGCTGCCTTGCA
CAACAACCAATGGCTCACCTCCCTCTCACAGGCTGCCTGAGCACACTCTTCCTGCAAGCAGCTGAGACCTTTGTGGAGTC
TGAGCTGCCACTGAGCTGGGCAAACTGGCTATGCAGCTACCTTCGGGGACTCTGGGCCTGGCTAGTGGTACTGTTGGCCA
CTTTTGTGGAGGCAGCACTATGTGCCTGGTATTTGATCGCTTTCCCACCAGAGGTGGTGACAGACTGGTCAGTGCTGCCC
ACAGAGGTACTGGAGCACTGCCACGTGCGTTCCTGGGTCAGCCTGGGCTTGGTGCACATCACCAATGCAATGTTAGCTTT
CCTCTGCTTTCTGGGCACTTTCCTGGTACAGAGCCAGCCTGGCCGCTACAACCGTGCCCGTGGTCTCACCTTCGCCATGC
TAGCTTATTTCATCACCTGGGTCTCTTTTGTGCCCCTCCTGGCCAATGTGCAGGTGGCCTACCAGCCAGCTGTGCAGATG
GGTGCTATCCTAGTCTGTGCCCTGGGCATCCTGGTCACCTTCCACCTGCCCAAGTGCTATGTGCTTCTTTGGCTGCCAAA
GCTCAACACCCAGGAGTTCTTCCTGGGAAGGAATGCCAAGAAAGCAGCAGATGAGAACAGTGGCGGTGGTGAGGCAGCTC
AGGGACACAATGAATGA

>mT1R3 (amino acid; SEQ ID NO: 8)
MPALAIMGLSLAAFLELGMGASLCLSQQFKAQGDYILGGLFPLGSTEEATLNQRTQPNSIPCNRFSPLGLFLAMAMKMAV
EEINNGSALLPGLRLGYDLFDTCSEPVVTMKSSLMFLAKVGSQSIAAYCNYTQYQPRVLAVIGPHSSELALITGKFFSFF
LMPQVSYSASMDRLSDRETFPSFFRTVPSDRVQLQAVVTLLQNFSWNWVAALGSDDDYGREGLSIFSSLANARGICIAHE
GLVPQHDTSGQQLGKVLDVLRQVNQSKVQVVVLFASARAVYSLFSYSIHHGLSPKVWVASESWLTSDLVMTLPNIARVGT
VLGFLQRGALLPEFSHYVETHLALAADPAFCASLNAELDLEEHVMGQRCPRCDDIMLQNLSSGLLQNLSAGQLHHQIFAT
YAAVYSVAQALHNTLQCNVSHCHVSEHVLPWQLLENMYNMSFHARDLTLQFDAEGNVDMEYDLKMWVWQSPTPVLHTVGT
FNGTLQLQQSKMYWPGNQVPVSQCSRQCKDGQVRRVKGFHSCCYDCVDCKAGSYRKHPDDFTCTPCNQDQWSPEKSTACL
PRRPKFLAWGEPVVLSLLLLLCLVLGLALAALGLSVHHWDSPLVQASGGSQFCFGLICLGLFCLSVLLFPGRPSSASCLA
QQPMAHLPLTGCLSTLFLQAAETFVESELPLSWANWLCSYLRGLWAWLVVLLATFVEAALCAWYLIAFPPEVVTDWSVLP
TEVLEHCHVRSWVSLGLVHITNAMLAFLCFLGTFLVQSQPGRYNRARGLTFAMLAYFITWVSFVPLLANVQVAYQPAVQM
GAILVCALGILVTFHLPKCYVLLWLPKLNTQEFFLGRNAKKAADENSGGGEAAQGHNE.

>rT1R1 (nucleic acid; SEQ ID NO: 16)
ATGCTCTTCTGGGCTGCTCACCTGCTGCTCAGCCTGCAGTTGGTCTACTGCTGGGCTTTCAGCTGCCAAAGGACAGAGTC
CTCTCCAGGCTTCAGCCTTCCTGGGGACTTCCTCCTTGCAGGTCTGTTCTCCCTCCATGGTGACTGTCTGCAGGTGAGAC
ACAGACCTCTGGTGACAAGTTGTGACAGGCCCGACAGCTTCAACGGCCATGGCTACCACCTCTTCCAAGCCATGCGGTTC
ACTGTTGAGGAGATAAACAACTCCTCGGCCCTGCTTCCCAACATCACCCTGGGGTATGAGCTGTACGACGTGTGCTCAGA
ATCTGCCAATGTGTATGCCACCCTGAGGGTGCTTGCCCTGCAAGGGCCCCGCCACATAGAGATACAGAAAGACCTTCGCA
ACCACTCCTCCAAGGTGGTGGCCTTCATCGGGCCTGACAACACTGACCACGCTGTCACTACCGCTGCCTTGCTGGGTCCT
TTCCTGATGCCCCTGGTCAGCTATGAGGCAAGCAGCGTGGTACTCAGTGCCAAGCGCAAGTTCCCGTCTTTCCTTCGTAC
CGTCCCCAGTGACCGGCACCAGGTGGAGGTCATGGTGCAGCTGCTGCAGAGTTTTGGGTGGGTGTGGATCTCGCTCATTG
GCAGCTACGGTGATTACGGGCAGCTGGGTGTGCAGGCGCTGGAGGAGCTGGCCGTGCCCCGGGGCATCTGCGTCGCCTTC
AAGGACATCGTGCCTTTCTCTGCCCGGGTGGGTGACCCGAGGATGCAGAGCATGATGCAGCATCTGGCTCAGGCCAGGAC
CACCGTGGTTGTGGTCTTCTCTAACCGGCACCTGGCTAGAGTGTTCTTCAGGTCCGTGGTGCTGGCCAACCTGACTGGCA
AAGTGTGGGTCGCCTCAGAAGACTGGGCCATCTCCACGTACATCACCAGCGTGACTGGGATCCAAGGCATTGGGACGGTG
CTCGGTGTGGCCGTCCAGCAGAGACAAGTCCCTGGGCTGAAGGAGTTTGAGGAGTCTTATGTCAGGGCTGTAACAGCTGC
TCCCAGCGCTTGCCCGGAGGGGTCCTGGTGCAGCACTAACCAGCTGTGCCGGGAGTGCCACACGTTCACGACTCGTAACA
TGCCCACGCTTGGAGCCTTCTCCATGAGTGCCGCCTACAGAGTGTATGAGGCTGTGTACGCTGTGGCCCACGGCCTCCAC
CAGCTCCTGGGATGTACTTCTGAGATCTGTTCCAGAGGCCCAGTCTACCCCTGGCAGCTTCTTCAGCAGATCTACAAGGT
GAATTTTCTTCTACATGAGAATACTGTGGCATTTGATGACAACGGGGACACTCTAGGTTACTACGACATCATCGCCTGGG
ACTGGAATGGACCTGAATGGACCTTTGAGATCATTGGCTCTGCCTCACTGTCTCCAGTTCATCTGGACATAAATAAGACA
AAAATCCAGTGGCACGGGAAGAACAATCAGGTGCCTGTGTCAGTGTGTACCACGGACTGTCTGGCAGGGCACCACAGGGT
GGTTGTGGGTTCCCACCACTGCTGCTTTGAGTGTGTGCCCTGCGAAGCTGGGACCTTTCTCAACATGAGTGAGCTTCACA
TCTGCCAGCCTTGTGGAACAGAAGAATGGGCACCCAAGGAGAGCACTACTTGCTTCCCACGCACGGTGGAGTTCTTGGCT
TGGCATGAACCCATCTCTTTGGTGCTAATAGCAGCTAACACGCTATTGCTGCTGCTGCTGGTTGGGACTGCTGGCCTGTT
TGCCTGGCATTTTCACACACCTGTAGTGAGGTCAGCTGGGGGTAGGCTGTGCTTCCTCATGCTGGGTTCCCTGGTGGCCG
GAAGTTGCAGCTTCTATAGCTTCTTCGGGGAGCCCACGGTGCCCGCGTGCTTGCTGCGTCAGCCCCTCTTTTCTCTCGGG
TTTGCCATCTTCCTCTCCTGCCTGACAATCCGCTCCTTCCAACTGGTCATCATCTTCAAGTTTTCTACCAAGGTGCCCAC
ATTCTACCGTACCTGGGCCCAAAACCATGGTGCAGGTCTATTCGTCATTGTCAGCTCCACGGTCCATTTGCTCATCTGTC
TCACATGGCTTGTAATGTGGACCCCACGACCCACCAGGGAATACCAGCGCTTCCCCCATCTGGTGATTCTCGAGTGCACA
GAGGTCAACTCTGTAGGCTTCCTGTTGGCTTTCACCCACAACATTCTCCTCTCCATCAGTACCTTCGTCTGCAGCTACCT
GGGTAAGGAACTGCCAGAGAACTATAATGAAGCCAAATGTGTCACCTTCAGCCTGCTCCTCAACTTCGTATCCTGGATCG
CCTTCTTCACCATGGCCAGCATTTACCAGGGCAGCTACCTGCCTGCGGTCAATGTGCTGGCAGGGCTGACCACACTGAGC
GGCGGCTTCAGCGGTTACTTCCTCCCCAAGTGCTATGTGATTCTCTGCCGTCCAGAACTCAACAATACAGAACACTTTCA
GGCCTCCATCCAGGACTACACGAGGCGCTGCGGCACTACCTGA

>rT1R1 (amino acid; SEQ ID NO: 3)
MLFWAAHLLLSLQLVYCWAFSCQRTESSPGFSLPGDFLLAGLFSLHGDCLQVRHRPLVTSCDRPDSFNGHGYHLFQAMRF
TVEEINNSSALLPNITLGYELYDVCSESANVYATLRVLALQGPRHIEIQKDLRNHSSKVVAFIGPDNTDHAVTTAALLGP
FLMPLVSYEASSVVLSAKRKFPSFLRTVPSDRHQVEVMVQLLQSFGWVWISLIGSYGDYGQLGVQALEELAVPRGICVAF
KDIVPFSARVGDPRMQSMMQHLAQARTTVVVFSNRHLARVFFRSVVLANLTGKVWVASEDWAISTYITSVTGIQGIGTV
LGVAVQQRQVPGLKEFEESYVRAVTAAPSACPEGSWCSTNQLCRECHTFTTRNMPTLGAFSMSAAYRVVEAVYAVAHGLH
QLLGCTSEICSRGPVYPWQLLQQIYKVNFLLHENTVAFDDNGDTLGYYDIIAWDWNGPEWTFEIIGSASLSPVHLDINKT
KIQWHGKNNQVPVSVCTTDCLAGHHRVVVGSHHCCFECVPCRAGTFLNMSELHICQPCGTEEWAPKESTTCFPRTVEFLA
WHEPISLVLIAANTLLLLLLVGTAGLFAWHFHTPVVRSAGGRLCFLMLGSLVAGSCSFYSFFGEPTVPACLLRQPLFSLG
FAIFLSCLTIRSFQLVIIFKFSTKVPTFYRTWAQNHGAGLFVIVSSTVHLLICLTWLVMWTPRPTREYQRFPHLVILECT
EVNSVGFLLAFTHNILLSISTFVCSYLGKELPENYNEAKCVTFSLLLNFVSWIAFFTMASIYQGSYLPAVNVLAGLTTLS
GGFSGYFLPKCYVILCRPELNNTEHFQASIQDYTRRCGTT.

>rT1R2 (Nucleic Acid; SEQ ID NO: 17)
ATGGGTCCCCAGGCAAGGACACTCTGCTTGCTGTCTCTCCTGCTGCATGTTCTGCCTAAGCCAGGCAAGCTGGTAGAGAA

CTCTGACTTCCACCTGGCCGGGGACTACCTCCTGGGTGGCCTCTTTACCCTCCATGCCAACGTGAAGAGCATCTCCCACC
TCAGCTACCTGCAGGTGCCCAAGTGCAATGAGTTCACCATGAAGGTGTTGGGCTACAACCTCATGCAGGCCATGCGTTTC
GCTGTGGAGGAGATCAACAACTGTAGCTCCCTGCTACCCGGCGTGCTGCTCGGCTACGAGATGGTGGATGTCTGTTACCT
CTCCAACAATATCCACCCTGGGCTCTACTTCCTGGCACAGGACGACGACCTCCTGCCCATCCTCAAAGACTACAGCCAGT
ACATGCCCCCACGTGGTGGCTGTCATTGGCCCCGACAACTCTGAGTCCGCCATTACCGTGTCCAACATTCTCTCTCATTTC
CTCATCCCACAGATCACATACAGCGCCATCTCCGACAAGCTGCGGGACAAGCGGCACTTCCCTAGCATGCTACGCACAGT
GCCCAGCGCCACCCACCACATCGAGGCCATGGTGCAGCTGATGGTTCACTTCCAATGGAACTGGATTGTGGTGCTGGTGA
GCGACGACGATTACGGCCGCGAGAACAGCCACCTGTTGAGCCAGCGTCTGACCAAAACGAGCGACATCTGCATTGCCTTC
CAGGAGGTTCTGCCCATACCTGAGTCCAGCCAGGTCATGAGGTCCGAGGAGCAGAGACAACTGGACAACATCCTGGACAA
GCTGCGGCGGACCTCGGCGCGCGTCGTGGTGGTGTTCTCGCCCGAGCTGAGCCTGTATAGCTTCTTTCACGAGGTGCTCC
GCTGGAACTTCACGGGTTTTGTGTGGATCGCCTCTGAGTCCTGGGCTATCGACCCAGTTCTGCATAACCTCACGGAGCTG
CGCCACACGGGTACTTTTCTGGGCGTCACCATCCAGAGGGTGTCCATCCCTGGCTTCAGTCAGTTCCGAGTGCGCCGTGA
CAAGCCAGGGTATCCCGTGCCTAACACGACCAACCTGCGGACGACCTGCAACCAGGACTGTGACGCCTGCTTGAACACCA
CCAAGTCCTTCAACAACATCCTTATACTTTCGGGGGAGCGCGTGGTCTACAGCGTGTACTCGGCAGTTTACGCGGTGGCC
CATGCCCTCCACAGACTCCTCGGCTGTAACCGGGTCCGCTGCACCAAGCAAAAGGTCTACCCGTGGCAGCTACTCAGGGA
GATCTGGCACGTCAACTTCACGCTCCTGGGTAACCGGCTCTTCTTTGACCAACAAGGGGACATGCCGATGCTCTTGGACA
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ATCCTCTCGTGCCACCCTAACTACCGCAACGGGCTACTGTTCAACACCAGCATGGACTTGCTGCTGTCTGTGCTGGGTTT
CAGCTTCGCTTACATGGGCAAGGAGCTGCCCACCAACTACAACGAAGCCAAGTTCATCACTCTCAGCATGACCTTCTCCT
TCACCTCCTCCATCTCCCTCTGCACCTTCATGTCTGTGCACGACGGCGTGCTGGTCACCATCATGGACCTCCTGGTCACT
GTGCTCAACTTCCTGGCCATCGGCTTGGGATACTTTGGCCCCAAGTGTTACATGATCCTTTTCTACCCGGAGCGCAACAC
CTCAGCCTATTTCAATAGCATGATCCAGGGCTACACCATGAGGAAGAGC

>rT1R2 (Amino Acid; SEQ ID NO: 6)
MGPQARTLCLLSLLLHVLPKPGKLVENSDFHLAGDYLLGGLFTLHANVKSISHLSYLQVPKCNEFTMKVLGYNLMQAMRF
AVEEINNCSSLLPGVLLGYEMVDVCYLSNNIHPGLYFLAQDDDLLPILKDYSQYMPHVVAVIGPDNSESAITVSNILSHF
LIPQITYSAISDKLRDKRHFPSMLRTVPSATHHIEAMVQLMVHFQWNWIVVLVSDDDYGRENSHLLSQRLTKTSDICIAF
QEVLPIPESSQVMRSEEQRQLDNILDKLRRTSARVVVVFSPELSLYSFFHEVLRWNFTGFVWIASESWAIDPVLHNLTEL
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HALHRLLGCNRVRCTKQKVYPWQLLREIWHVNFTLLGNRLFFDQQGDMPMLLDIIQWQWDLSQNPFQSIASYSPTSKRLT
YINNVSWYTPNNTVPVSMCSKSCQPGQMKKSVGLHPCCFECLDCMPGTYLNRSADEFNCLSCPGSMWSYKNDITCFQRRP
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FTVCFSICLSCITVRSFQIVCVFKMARRLPSAYSFWMRYHGPYVFVAFITAIKVALVVGNMLATTINPIGRTDPDDPNIM
ILSCHPNYRNGLLFNTSMDLLLSVLGFSFAYMGKELPTNYNEAKFITLSMTFSFTSSISLCTFMSVHDGVLVTIMDLLVT
VLNFLAIGLGYFGPKCYMILFYPERNTSAYFNSMIQGYTMRKS

>rT1R3 (Nucleic Acid; SEQ ID NO: 18)
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GAACACAGCCCAACGGCATCCTATGTACCAGGTTCTCGCCCCCTTGGTTTGTTCCTGGCCATGGCTATGAAGATGGCTGTA
GAGGAGATCAACAATGGATCTGCCTTGCTCCCTGGGCTGCGACTGGGCTATGACCTGTTTGACACATGCTCAGAGCCAGT
GGTCACCATGAAGCCCAGCCTCATGTTCATGGCCAAGGTGGGAAGTCAAAGCATTGCTGCCTACTGCAACTACACACAGT
ACCAACCCCGTGTGCTGGCTGTCATTGGTCCCCACTCATCAGAGCTTGCCCTCATTACAGGCAAGTTCTTCAGCTTCTTC
CTCATGCCACAGGTCAGCTATAGTGCCAGCATGGATCGGCTAAGTGACCGGGAAACATTTCCATCCTTCTTCCGCACAGT
GCCCAGTGACCGGGTGCAGCTGCAGGCCGTTGTGACACTGTTGCAGAATTTCAGCTGGAACTGGGTGGCTGCCTTAGGTA
GTGATGATGACTATGGCCGGGAAGGTCTGAGCATCTTTTCTGGTCTGGCCAACTCACGAGGTATCTGCATTGCACACGAG
GGCCTGGTGCCACAACATGACACTAGTGGCCAACAATTGGGCAAGGTGGTGGATGTGCTACGCCAAGTGAACCAAAGCAA
AGTACAGGTGGTGGTGCTGTTTGCATCTGCCCGTGCTGTCTACTCCCTTTTTAGCTACAGCATCCTTCATGACCTCTCAC
CCAAGGTATGGGTGGCCAGTGAGTCCTGGCTGACCTCTGACCTGGTCATGACACTTCCCAATATTGCCCGTGTGGGCACT
GTTCTTGGGTTTCTGCAGCGCGGTGCCCTACTGCCTGAATTTTCCCATTATGTGGAGACTCGCCTTGCCCTAGCTGCTGA
CCCAACATTCTGTGCCTCCCTGAAAGCTGAGTTGGATCTGGAGGAGCGCGTGATGGGGCCACGCTGTTCACAATGTGACT
ACATCATGCTACAGAACCTGTCATCTGGGCTGATGCAGAACCTATCAGCTGGGCAGTTGCACCACCAAATATTTGCAACC
TATGCAGCTGTGTACAGTGTGGCTCAGGCCCTTCACAACACCCTGCAGTGCAATGTCTCACATTGCCACACATCAGAGCC
TGTTCAACCCTGGCAGCTCCTGGAGAACATGTACAATATGAGTTTCCGTGCTCGAGACTTGACACTGCAGTTTGATGCCA
AAGGGAGTGTAGACATGGAATATGACCTGAAGATGTGGGTGTGGCAGAGCCCTACACCTGTACTACATACTGTAGGCACC
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CTCTTGTGGAGGCTGCACTATGTGCCTGGTACTTGATGGCTTTCCCTCCAGAGGTGGTGACAGATTGGCAGGTGCTGCCC
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GGTGCTATCTTATTCTGTGCCCTGGGCATCCTGGCCACCTTCCACCTGCCCAAATGCTATGTACTTCTGTGGCTGCCAGA
GCTCAACACCCAGGAGTTCTTCCTGGGAAGGAGCCCCAAGGAAGCATCAGATGGGAATAGTGGTAGTAGTGAGGCAACTC
GGGGACACAGTGAATGA

>rT1R3 (Amino Acid; SEQ ID NO: 9)
MPGLAILGLSLAAFLELGMGSSLCLSQQFKAQGDYILGGLFPLGTTEEATLNQRTQPNGILCTRFSPLGLFLAMAMKMAV
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LMPQVSYSASMDRLSDRETFPSFFRTVPSDRVQLQAVVTLLQNFSWNWVAALGSDDDYGREGLSIFSGLANSRGICIAHE
GLVPQHDTSGQQLGKVVDVLRQVNQSKVQVVVLFASARAVYSLFSYSILHDLSPKVWVASESWLTSDLVMTLPNIARVGT
VLGFLQRGALLPEFSHYVETRLALAADPTFCASLKAELDLEERVMGPRCSQCDYIMLQNLSSGLMQNLSAGQLHHQIFAT
YAAVYSVAQALHNTLQCNVSHCHTSEPVQPWQLLENMYNMSFRARDLTLQFDAKGSVDMEYDLKMWVWQSPTPVLHTVGT
FNGTLQLQHSKMYWPGNQVPVSQCSRQCKDGQVRRVKGFHSCCYDCVDCKAGSYRKHPDDFTCTPCGKDQWSPEKSTTCL
PRRPKFLAWGEPAVLSLLLLLCLVLGLTLAALGLFVHYWDSPLVQASGGSLFCFGLICLGLFCLSVLLFPGRPRSASCLA
QQPMAHLPLTGCLSTLFLQAAEIFVESELPLSWANWLCSYLRGPWAWLVVLLATLVEAALCAWYLMAFPPEVVTDWQVLP
TEVLEHCRMRSWVSLGLVHITNAVLAFLCFLGTFLVQSQPGRYNRARGLTFAMLAYFIIWVSFVPLLANVQVAYQPAVQM
GAILFCALGILATFHLPKCYVLLWLPELNTQEFFLGRSPKEASDGNSGSSEATRGHSE.

WHAT IS CLAIMED IS:

1. An isolated or recombinant polypeptide that comprises one or more of the following:

(a.) an amino acid sequence or subsequence that is at least 75% identical to SEQ ID NO. 1, SEQ ID NO.4, or SEQ ID NO.7 as determined by BLASTP using default parameters;

(b.) an amino acid sequence or subsequence that comprises one or more domains of an hT1R1 polypeptide, an hT1R2 polypeptide, or an hT1R3 polypeptide, wherein the hT1R1 polypeptide, the hT1R2 polypeptide, or the hT1R3 polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO.4 and SEQ ID NO.7;

(c.) an amino acid sequence or subsequence that is at least 75% identical to a domain encoded by SEQ ID NO. 1, SEQ ID NO.4 or SEQ ID NO.7 as determined by BLASTP using default parameters, wherein the domain is selected from the group consisting of: an amino-terminal extracellular domain; an extracellular domain located between TM2 and TM3, between TM4 and TM5, or between TM6 and TM7; a transmembrane (TM) domain; an intracellular domain located between TM1 and TM2, between TM3 and TM4, or between TM5 and TM6; and a carboxyl-terminal intracellular domain;

(d.) an amino acid sequence or subsequence that is specifically bound by an antibody that specifically binds to an amino acid selected from the group consisting of SEQ ID NO. 1, SEQ ID NO.4, and SEQ ID NO.7, wherein the antibody is not specifically bound by an amino acid selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 8, and SEQ ID NO. 9;

(e.) an amino acid sequence or subsequence that is encoded by SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO 12, or a complementary sequence thereof;

(f.) an amino acid sequence or subsequence that is encoded by a first nucleic acid that specifically hybridizes to a second nucleic acid, wherein the second nucleic acid is selected from the group consisting of: SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO 12, or a complement thereof, under stringent conditions, wherein the first nucleic acid hybridizes to the second nucleic acid under said stringent conditions with at least 5x an affinity that the

first nucleic acid hybridizes to a third nucleic acid selected from the group consisting of: an mT1R1 nucleic acid, an rT1R1 nucleic acid, an mT1R2 nucleic acid, an rT1R2 nucleic acid, an mT1R3 nucleic acid and a rT1R3 nucleic acid; or,

(g.) an amino acid sequence or subsequence corresponding to a conservative variation an amino acid sequence or subsequence of any one of (a.)-(f.).

2. The isolated or recombinant polypeptide of claim **1**, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO. 1, SEQ ID NO.4, SEQ ID NO.7, or a conservative variation thereof.

3. The isolated or recombinant polypeptide of claim **1**, wherein the polypeptide comprises a mature hT1R1 protein, a mature hT1R2 protein, or a mature hT1R3 protein.

4. The isolated polypeptide of claim **1**, wherein the domain of (b.) is selected from the group consisting of:

an amino-terminal extracellular domain;

an extracellular domain located between TM2 and TM3, between TM4 and TM5, or between TM6 and TM7;

a transmembrane (TM) domain;

an intracellular domain located between TM1 and TM2, between TM3 and TM4, or between TM5 and TM6; and

a carboxyl-terminal intracellular domain.

5. The polypeptide of claim **1**, wherein the polypeptide is a heteromer.

6. The polypeptide of claim **1**, wherein the polypeptide is a homomultimer.

7. The polypeptide of claim **1**, wherein the polypeptide is a heteromer that comprises more than one polypeptide selected from the group consisting of SEQ ID NO. 1, SEQ ID NO.4, and SEQ ID NO.7, or a conservative variation thereof.

8. An isolated or recombinant nucleic acid that encodes the isolated or recombinant polypeptide of claim **1**, wherein the polypeptide is a substantially full-length a polypeptide, or wherein the nucleic acid is capable of rescuing function of a mutant or recombinant cell that is defective with respect to hT1R1, hT1R2 or hT1R3.

9. The isolated or recombinant nucleic acid of claim **8**, wherein the nucleic acid is a DNA.

10. The isolated or recombinant nucleic acid of claim **8**, wherein the nucleic acid is a cDNA.

11. The isolated or recombinant nucleic acid of claim **8**, wherein the nucleic acid is an RNA.

12. The isolated or recombinant nucleic acid of claim **8**, wherein the nucleic acid is a coding nucleic acid that encodes an amino acid sequence selected from the group consisting of: SEQ ID NO. 1, SEQ ID NO. 4, and SEQ ID NO. 7, or a conservative variation thereof; or wherein the isolated or recombinant nucleic acid is a complementary nucleic acid that is complementary to the coding nucleic acid.

13. The isolated or recombinant nucleic acid of claim **8**, wherein the nucleic acid comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, and a complementary sequence thereof.

14. The isolated or recombinant nucleic acid of claim **8**, wherein the nucleic acid comprises or is coded within an expression vector.

15. An hT1R2 nucleic acid that hybridizes under stringent conditions to a first nucleic acid comprising the first two exons from nucleotide sequence of SEQ ID NO. 11 in the region of the first two exons, or to a complement thereof, wherein the stringent conditions are selected such that the hT1R2 nucleic acid preferentially hybridizes to the first nucleic acid as compared to a mT1R2 nucleic acid or complement thereof, or to an rT1R2 nucleic acid or complement thereof.

16. The hT1R2 nucleic acid of claim **15**, wherein the nucleic acid encodes a substantially full length hT1R2 polypeptide.

17. The hT1R2 nucleic acid of claim **15**, wherein the hT1R2 nucleic acid is a cDNA or an RNA.

18. The hT1R2 nucleic acid of claim **15**, wherein the hT1R2 nucleic acid comprises or is coded within an expression vector.

19. The hT1R2 nucleic acid of claim **15**, wherein the hT1R2 nucleic acid encodes a polypeptide comprising the sequence set forth at SEQ ID NO: 4.

20. A cDNA or mRNA that encodes the isolated or recombinant polypeptide of claim **1**.

21. An expression vector encoding the cDNA or mRNA of claim **20**.

22. An antibody or fragment thereof which specifically binds the isolated or recombinant polypeptide of claim **1**.

23. An antibody fragment according to claim **22**, wherein the antibody fragment is an Fab or F(ab')2 fragment.

24. An antibody according to claim **22** wherein the antibody is a polyclonal antibody.

25. An antibody according to claim **22** which is a monoclonal antibody.

26. An antibody according to claim **22**, wherein the antibody does not bind to any protein selected from the group consisting of: mT1R1, mT1R2, mT1R3, rT1R1, rT1R2 and rT1R3.

27. An expression vector that encodes a polypeptide of claim **1**.

28. A cell comprising the expression vector of claim **27**.

29. A biosensor comprising the polypeptide of claim **1**.

30. A method for producing a recombinant or isolated polypeptide, comprising:

(a.) culturing a cell comprising an expression vector encoding the recombinant or isolated polypeptide of claim **1**, under conditions suitable for expression of the isolated or recombinant polypeptide; and,

(b.) purifying the polypeptide such that the polypeptide is enriched at least 5X as compared to the polypeptide present in step (a).

31. The method of claim **30**, wherein (b.) comprises purifying the polypeptide such that the polypeptide is enriched at least 100 X as compared to the polypeptide present in step (a.).

32. An isolated or recombinant polypeptide made by the method of claim **30**.

33. A method of identifying compounds which bind to and/or modulate an activity of the isolated or recombinant polypeptide of claim **1**, the method comprising:

(a.) contacting a biological sample comprising the isolated or recombinant polypeptide with a test compound; and,

(b.) detecting binding and/or modulation of the activity of the polypeptide by the compound, thereby identifying a compound which binds to and/or modulates the activity of the polypeptide.

34. The method of claim **33**, wherein step (b.) includes detecting binding of an antibody to the isolated or recombinant polypeptide.

35. The method of claim **33**, wherein step (b.) includes detecting a signal produced by the isolated or recombinant polypeptide.

36. The method of claim **35**, wherein the signal is a conformation-dependent signal, wherein a conformation of the isolated or recombinant polypeptide is modified by binding of the test compound to the isolated or recombinant polypeptide.

37. The method of claim **33**, wherein the biological sample comprises a cell which expresses the recombinant polypeptide.

38. The method of claim **33**, wherein detecting binding comprises one or more of: a Ca^{2+} flux assay, a cAMP assay, a GTPgammaS binding assay, a melanophore assay, a phospholipase C assay, a beta-arrestin FRET assay, and a transcriptional reporter assay.

39. The method of claim **33**, wherein the transcriptional reporter assay comprises detecting an activity of one or response element selected from the group consisting of: a CRE, a SRE, an MRE, a TRE, an NFAT, and an NFkB-response element, wherein the response element directs expression of an operably coupled reporter gene.

40. The method of claim **33**, wherein the biological sample comprises a biosensor.

41. The method of claim **41**, wherein the biosensor comprises a Chem-FET.

42. A method of identifying compounds which bind to and/or modulate an activity of a polypeptide comprising hT1R1, hT1R2 or hT1R3, the method comprising:

(a.) contacting a biological sample comprising hT1R1, hT1R2 or hT1R3 with a test compound; and,

(b.) detecting binding and/or modulation of the activity of the polypeptide by the compound, thereby identifying a compound which binds to and/or modulates the activity of the polypeptide.

43. The method of claim **42**, wherein step (b.) includes detecting binding of an antibody to the isolated or recombinant polypeptide.

44. The method of claim **42**, wherein step (b.) includes detecting a signal produced by the isolated or recombinant polypeptide.

45. The method of claim **42**, wherein the signal is a conformation-dependent signal, wherein a conformation of the isolated or recombinant polypeptide is modified by binding of the test compound to the isolated or recombinant polypeptide.

46. The method of claim **42**, wherein the biological sample comprises a cell which expresses the recombinant polypeptide.

47. The method of claim **42**, wherein detecting binding comprises one or more of: a Ca^{2+} flux assay, a cAMP assay, a GTPgammaS binding assay, a melanophore assay, a phospholipase C assay, a beta-arrestin FRET assay, and a transcriptional reporter assay.

48. The method of claim **47**, wherein the transcriptional reporter assay comprises detecting an activity of one or response element selected from the group consisting of: a CRE, a SRE, an MRE, a TRE, an NFAT, and an NFkB-response element, wherein the response element directs expression of an operably coupled reporter gene.

49. The method of claim **42**, wherein the biological sample comprises a biosensor.

50. The method of claim **49**, wherein the biosensor comprises a Chem-FET.

51. A method of rescuing a cell that has altered or missing T1R1, T1R2, or T1R3 function, comprising introducing a nucleic acid into the cell, wherein the nucleic acid encodes the recombinant polypeptide of claim **1**, and expressing recombinant polypeptide, thereby providing hT1R1, hT1R2, or hT1R3 function to the cell.

52. The method of claim **51**, wherein the cell is in cell culture.

53. The method of claim **51**, wherein the cell is in a tissue.

54. The method of claim **51**, wherein the cell is in a taste bud.

55. The method of claim **51**, wherein the cell is in taste bud in a mammal.

FIGURE 1

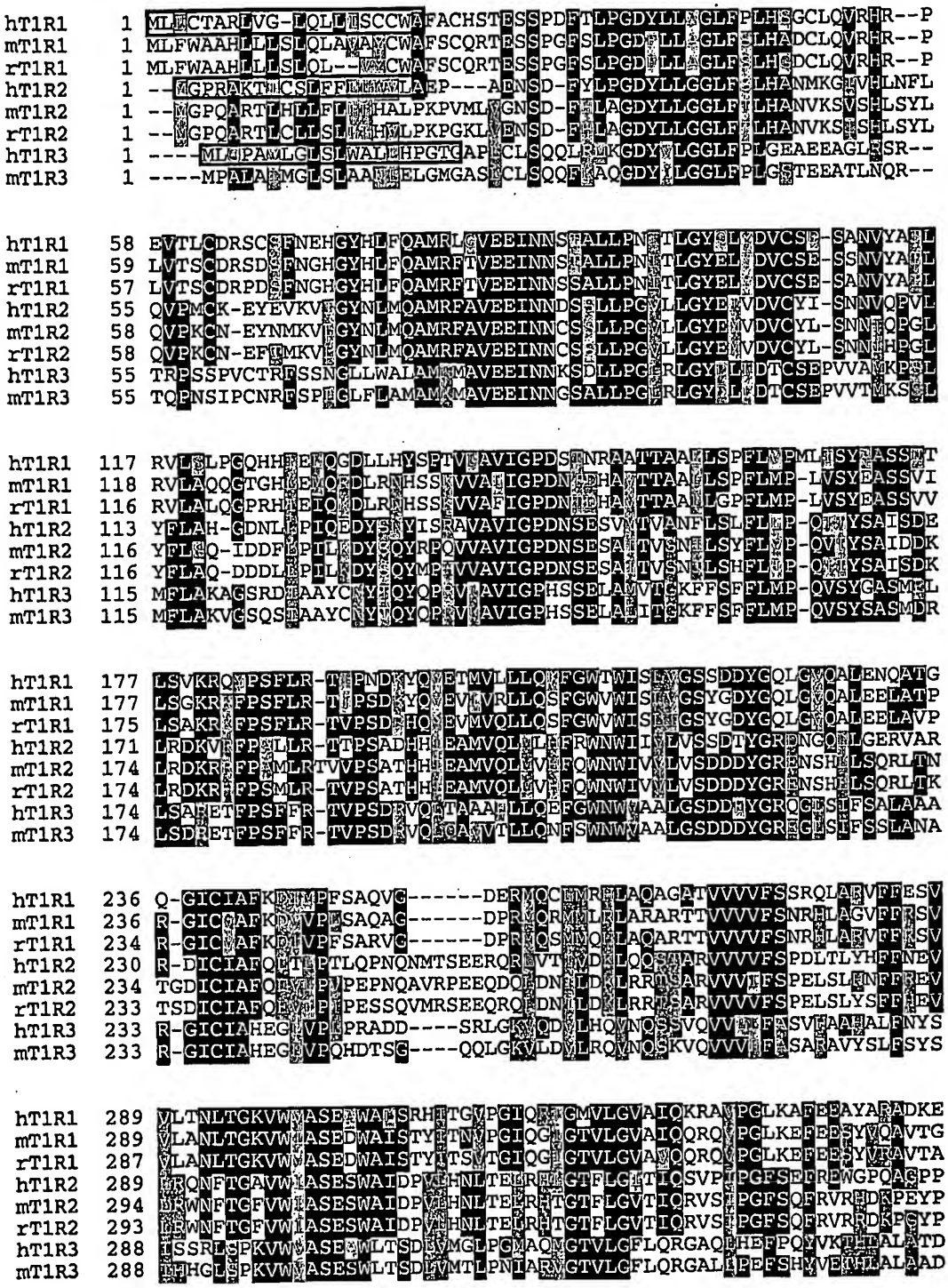

FIGURE 1 (CONT'D)

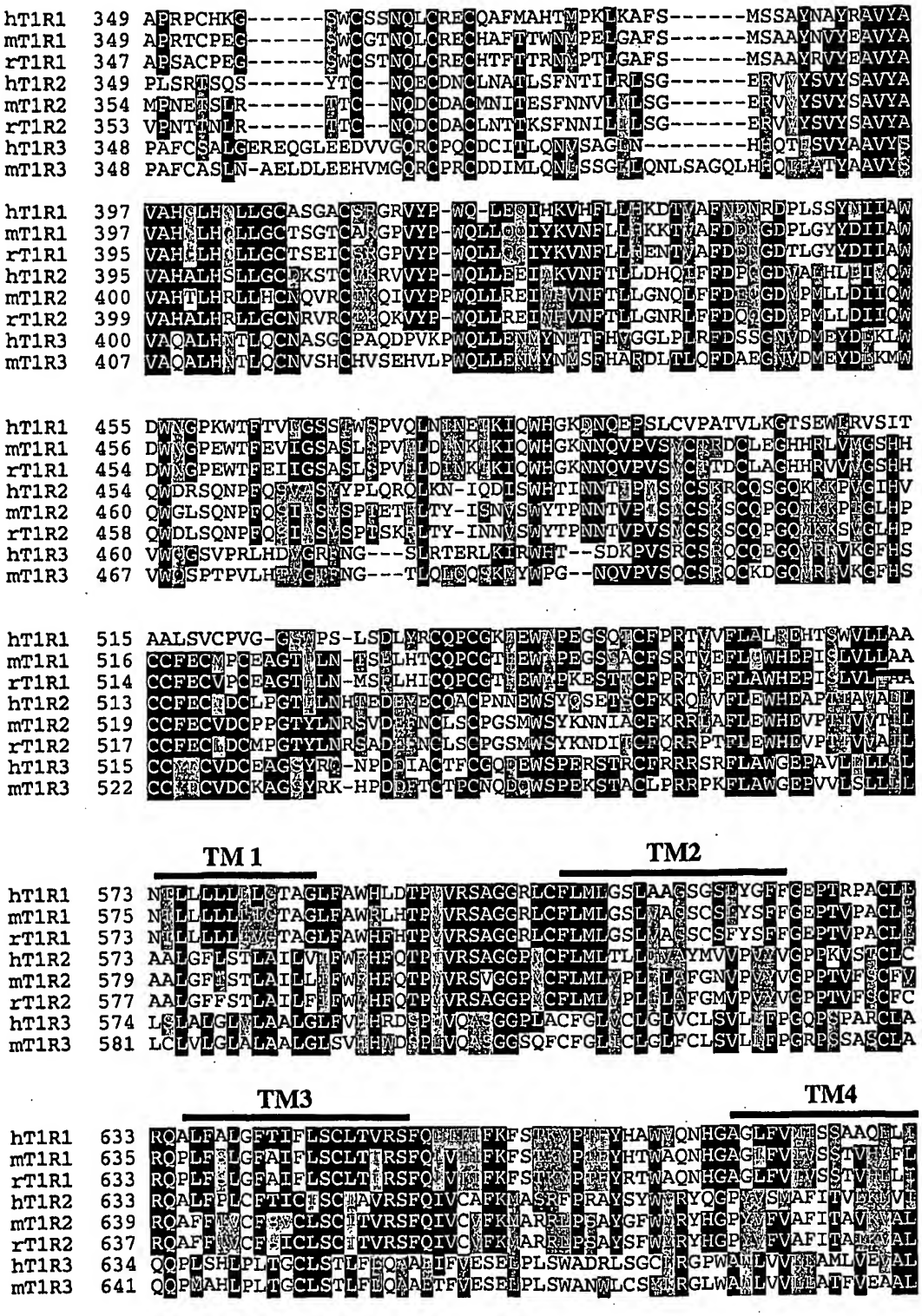

FIGURE 1 (CONT'D)

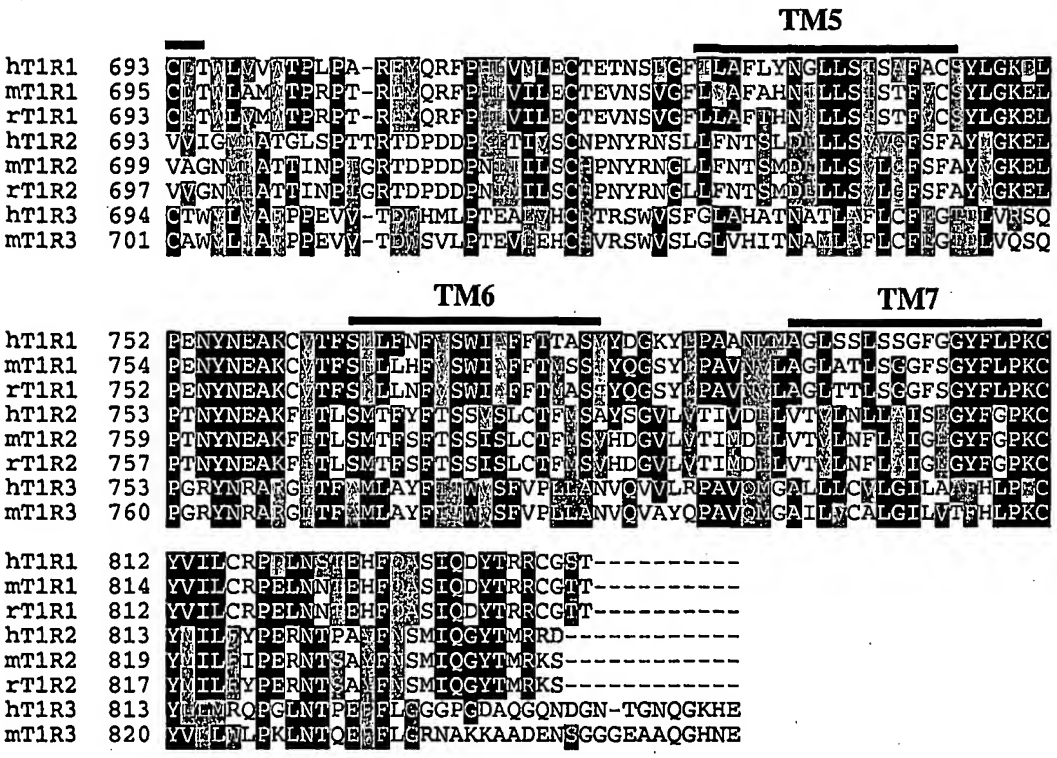

FIGURE 2A

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GACAGGTCTTGTAGCTTCAATGAGCATGGCTACCACCTCTTCCAGGCTATGCGGCTTGGGGT
TGAGGAGATAAACAACTCCACGGCCCTGCTGCCCAACATCACCCTGGGGTACCAGCTGTATG
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CACCACATAGAGCTCCAAGGAGACCTTCTCCACTATTCCCCTACGGTGCTGGCAGTGATTGG
GCCTGACAGCACCAACCGTGCTGCCACCACAGCCGCCCTGCTGAGCCCTTTCCTGGTGCCCA
TGCTTATTAGCTATGCGGCCAGCAGCGAGACGCTCAGCGTGAAGCGGCAGTATCCCTCTTTC
CTGCGCACCATCCCCAATGACAAGTACCAGGTGGAGACCATGGTGCTGCTGCTGCAGAAGTT
CGGGTGGACCTGGATCTCTCTGGTTGGCAGCAGTGACGACTATGGGCAGCTAGGGGTGCAGG
CACTGGAGAACCAGGCCACTGGTCAGGGGATCTGCATTGCTTTCAAGGACATCATGCCCTTC
TCTGCCCAGGTGGGCGATGAGAGGATGCAGTGCCTCATGCGCCACCTGGCCCAGGCCGGGGC
CACCGTCGTGGTTGTTTTTTCCAGCCGGCAGTTGGCCAGGGTGTTTTTCGAGTCCGTGGTGC
TGACCAACCTGACTGGCAAGGTGTGGGTCGCCTCAGAAGCCTGGGCCCTCTCCAGGCACATC
ACTGGGGTGCCCGGGATCCAGCGCATTGGGATGGTGCTGGGCGTGGCCATCCAGAAGAGGGC
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CTTGCCACAAGGGCTCCTGGTGCAGCAGCAATCAGCTCTGCAGAGAATGCCAAGCTTTCATG
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GCCTGCTAGGGAATACCAGCGCTTCCCCCATCTGGTGATGCTTGAGTGCACAGAGACCAACT
CCCTGGGCTTCATACTGGCCTTCCTCTACAATGGCCTCCTCTCCATCAGTGCCTTTGCCTGC
AGCTACCTGGGTAAGGACTTGCCAGAGAACTACAACGAGGCCAAATGTGTCACCTTCAGCCT
GCTCTTCAACTTCGTGTCCTGGATCGCCTTCTTCACCACGGCCAGCGTCTACGACGGCAAGT
ACCTGCCTGCGGCCAACATGATGGCTGGGCTGAGCAGCCTGAGCAGCGGCTTCGGTGGGTAT
TTTCTGCCTAAGTGCTACGTGATCCTCTGCCGCCCAGACCTCAACAGCACAGAGCACTTCCA
GGCCTCCATTCAGGACTACACGAGGCGCTGCGGCTCCACCTGA

FIGURE 2B

ATGGGGCCCAGGGCAAAGACCATCTGCTCCCTGTTCTTCCTCCTATGGGTCCTGGCTGAGCC
GGCTGAGAACTCGGACTTCTACCTGCCTGGGGATTACCTCCTGGGTGGCCTCTTCTCCCTCC
ATGCCAACATGAAGGGCATTGTTCACCTTAACTTCCTGCAGGTGCCCATGTGCAAGGAGTAT
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CAATGACAGCAGCCTGCTGCCTGGTGTGCTGCTGGGCTATGAGATCGTGGATGTGTGCTACA
TCTCCAACAATGTCCAGCCGGTGCTCTACTTCCTGGCACACGGGGACAACCTCCTTCCCATC
CAAGAGGACTACAGTAACTACATTTCCCGTGCGGTGGCTGTCATTGGCCCTGACAACTCCGA
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CCATCAGCGATGAGCTGCGAGACAAGGTGCGCTTCCCGGCTTTGCTGCGTACCACACCCAGC
GCCGACCACCACATCGAGGCCATGGTGCAGCTGATGCTGCACTTCCGCTGGAACTGGATCAT
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GCGCGTCGTGGTCGTGTTCTCGCCCGACCTGACCCTGTACCACTTCTTCAATGAGGTGCTGC
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CATCCCGGGCTTCAGTGAGTTCCGCGAGTGGGGCCCACAGGCTGGGCCGCCACCCCTCAGCA
GGACCAGCCAGAGCTATACCTGCAACCAGGAGTGCGACAACTGCCTGAACGCCACCTTGTCC
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GCCCGAATAACGAGTGGTCCTACCAGAGTGAGACCTCCTGCTTCAAGCGGCAGCTGGTCTTC
CTGGAATGGCATGAGGCACCCACCATCGCTGTGGCCCTGCTGGCCGCCCTGGGCTTCCTCAG
CACCCTGGCCATCCTGGTGATATTCTGGAGGCACTTCCAGACACCCATAGTTCGCTCGGCTG
GGGGCCCCATGTGCTTCCTGATGCTGACACTGCTGCTGGTGGCATACATGGTGGTCCCGGTG
TACGTGGGGCCGCCCAAGGTCTCCACCTGCCTCTGCCGCCAGGCCCTCTTTCCCCTCTGCTT
CACAATCTGCATCTCCTGTATCGCCGTGCGTTCTTTCCAGATCGTCTGCGCCTTCAAGATGG
CCAGCCGCTTCCCACGCGCCTACAGCTACTGGGTCCGCTACCAGGGGCCCTACGTCTCTATG
GCATTTATCACGGTACTCAAAATGGTCATTGTGGTAATTGGCATGCTGGCCACGGGCCTCAG
TCCCACCACCCGTACTGACCCCGATGACCCCAAGATCACAATTGTCTCCTGTAACCCCAACT
ACCGCAACAGCCTGCTGTTCAACACCAGCCTGGACCTGCTGCTCTCAGTGGTGGGTTTCAGC
TTCGCCTACATGGGCAAAGAGCTGCCCACCAACTACAACGAGGCCAAGTTCATCACCCTCAG
CATGACCTTCTATTTCACCTCATCCGTCTCCCTCTGCACCTTCATGTCTGCCTACAGCGGGG
TGCTGGTCACCATCGTGGACCTCTTGGTCACTGTGCTCAACCTCCTGGCCATCAGCCTGGGC
TACTTCGGCCCCAAGTGCTACATGATCCTCTTCTACCCGGAGCGCAACACGCCCGCCTACTT
CAACAGCATGATCCAGGGCTACACCATGAGGAGGGACTAG

FIGURE 2C

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CCCTGGGCGAGGCCGAGGAGGCTGGCCTCCGCAGCCGGACACGGCCCAGCAGCCCTGTGTGC
ACCAGGTTCTCCTCAAACGGCCTGCTCTGGGCACTGGCCATGAAAATGGCCGTGGAGGAGAT
CAACAACAAGTCGGATCTGCTGCCCGGGCTGCGCCTGGGCTACGACCTCTTTGATACGTGCT
CGGAGCCTGTGGTGGCCATGAAGCCCAGCCTCATGTTCCTGGCCAAGGCAGGCAGCCGCGAC
ATCGCCGCCTACTGCAACTACACGCAGTACCAGCCCCGTGTGCTGGCTGTCATCGGGCCCCA
CTCGTCAGAGCTCGCCATGGTCACCGGCAAGTTCTTCAGCTTCTTCCTCATGCCCCAGGTCA
GCTACGGTGCTAGCATGGAGCTGCTGAGCGCCCGGGAGACCTTCCCCTCCTTCTTCCGCACC
GTGCCCAGCGACCGTGTGCAGCTGACGGCCGCCGCGGAGCTGCTGCAGGAGTTCGGCTGGAA
CTGGGTGGCCGCCCTGGGCAGCGACGACGAGTACGGCCGGCAGGGCCTGAGCATCTTCTCGG
CCCTGGCCGCGGCACGCGGCATCTGCATCGCGCACGAGGGCCTGGTGCCGCTGCCCCGTGCC
GATGACTCGCGGCTGGGGAAGGTGCAGGACGTCCTGCACCAGGTGAACCAGAGCAGCGTGCA
GGTGGTGCTGCTGTTCGCCTCCGTGCACGCCGCCCACGCCCTCTTCAACTACAGCATCAGCA
GCAGGCTCTCGCCCAAGGTGTGGGTGGCCAGCGAGGCCTGGCTGACCTCTGACCTGGTCATG
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CCTTGTGCTGGCTGCTTTGGGGCTGTTCGTTCACCATCGGGACAGCCCACTGGTTCAGGCCT
CGGGGGGGCCCCTGGCCTGCTTTGGCCTGGTGTGCCTGGGCCTGGTCTGCCTCAGCGTCCTC
CTGTTCCCTGGCCAGCCCAGCCCTGCCCGATGCCTGGCCCAGCAGCCCTTGTCCCACCTCCC
GCTCACGGGCTGCCTGAGCACACTCTTCCTGCAGGCGGCCGAGATCTTCGTGGAGTCAGAAC
TGCCTCTGAGCTGGGCAGACCGGCTGAGTGGCTGCCTGCGGGGGCCCTGGGCCTGGCTGGTG
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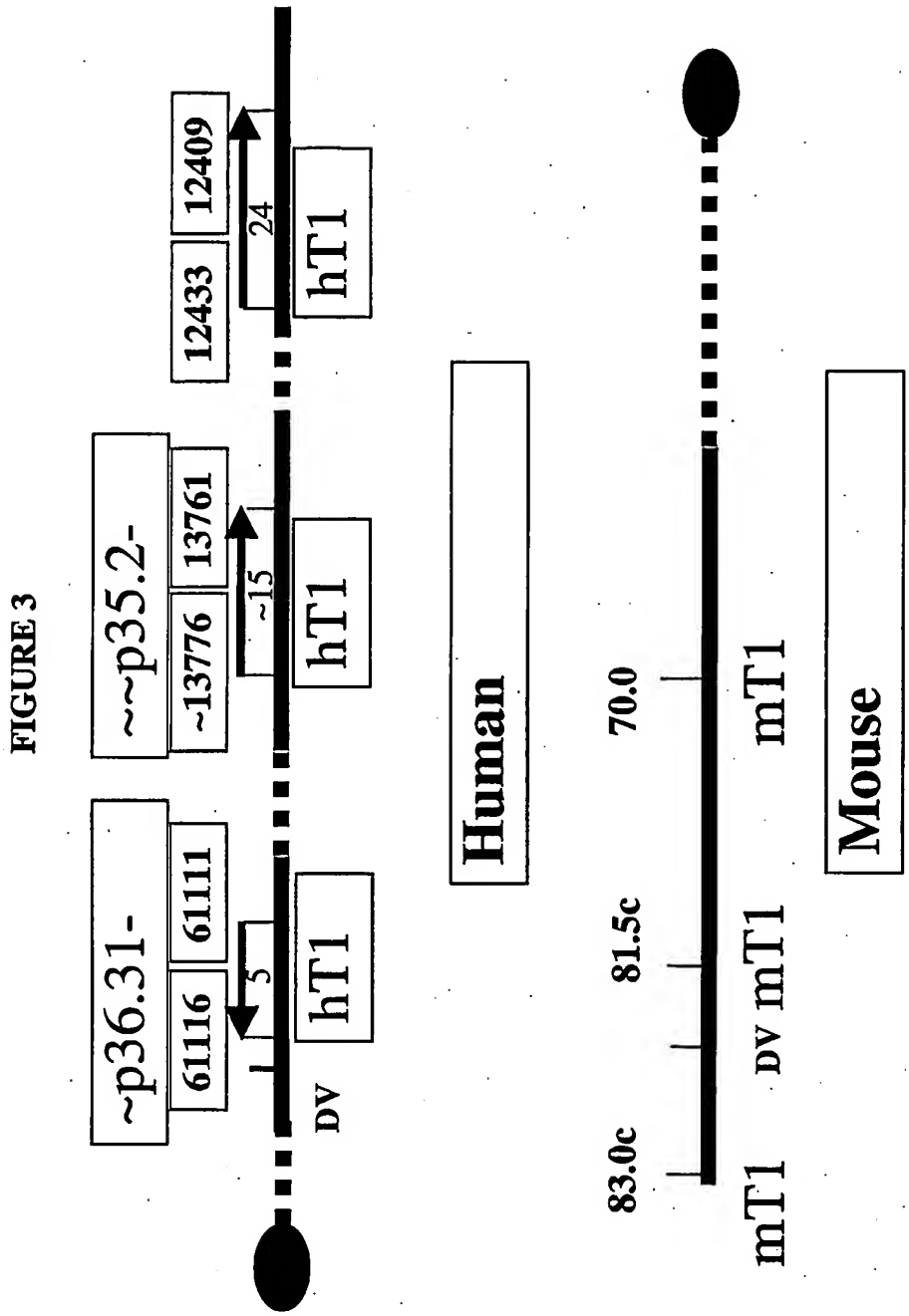
TCCTCAGGCCCGCCGTGCAGATGGGCGCCCTCCTGCTCTGTGTCCTGGGCATCCTGGCTGCC
TTCCACCTGCCCAGGTGTTACCTGCTCATGCGGCAGCCAGGGCTCAACACCCCCGAGTTCTT
CCTGGGAGGGGGCCCTGGGGATGCCCAAGGCCAGAATGACGGGAACACAGGAAATCAGGGGA
AACATGAGTGA

FIGURE 3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81.5c
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